REMARKS

Summary of Personal Interview with Examiner

Applicants thank the Examiner for the courtesy of the telephonic interview conducted on September 13, 2006 during which the foregoing amendments to the claims and the outstanding rejections were discussed

Amendments to the Claims

Claims 1, 4, 9-14, 17, 25, 26, 28, 29, 31-33, 39 and 40 were pending in the application as of the issuance of the present Office Action. Claims 1, 4-6, 9-14, 17, 25, 26, 28, 29, 31-33, 39 and 40 stand rejected. In the Amendment to the Claims spanning pages 2 to 5 of this paper, claims 1, 13, 25, 26, 31, 33 and 40 have been amended and claim 32 has been canceled, without prejudice. Accordingly, upon entry of the amendments presented herein, claims 1, 4, 9-14, 17, 25, 26, 28, 29, 31, 33, 39 and 40 will remain pending in this application.

Support for the amendments to the claims can be found throughout the specification and the claims as originally filed. Specifically, support for the amendments to claims 25, 26, 31 and 33 can be found throughout the specification, for example, on page 10, line 1 to page 11, line 34 of the specification, and in the claims as originally filed, for example, claims 31-33 .

No new matter has been added by these claim amendments. Any cancellation and/or amendments to the claims have been made solely in the interest of expediting examination and in no way acquiescing to the validity of the Examiner's rejections. Applicants reserve the right to pursue the claims as originally filed in one or more further applications.

Withdrawn Claim Rejections

Applicants gratefully acknowledge the Examiner's withdrawal of the following rejections:

a) the previous rejection of claims 12-14 under 35 U.S.C. §101 as being directed toward non-statutory subject matter; and

b) the previous rejection of claims 5, 6, 9-14, 17 and 39 under 35 U.S.C. §112, first paragraph as failing to meet the enablement requirement.

Priority

Applicants acknowledge that the instant claims are granted the priority date of June 23, 2000, the filing date of the instant application. Additionally, Applicants note that certified copies of the foreign German patent applications will be filed prior to the issuance of a patent, at which time Applicants will request grant of the foreign priority, if appropriate.

Rejection of Claims 1, 9-14, 17, 25, 26, 28, 29, 31-33 and 40 Under 35 U.S.C. § 112

The Examiner has rejected claims 1, 9-14, 17, 25, 26, 28, 29, 31-33 and 40 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Examiner is of the opinion that

> [c]laim 1 recites the phrase 'less than about 5 kb of nucleotide sequences' which renders the claim vague and indefinite. The metes and bounds of the claim are not certain since the meaning of the phrase is not clear. It is uncertain whether 5 kb of nucleotide sequences actually flanks SEQ ID NO:1.
>
> No patentable weight is given to this limitation since the metes and bounds of the limitation are not known. The phrase 'less than about' is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree of 'less than about', and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

In order to expedite examination, but in no way acquiescing to the validity of the Examiner's rejection, Applicants have amended claim 1 to recite "...less than 5 kb..." and further claim 40 to recite "...less than 4 kb...," thereby rendering the foregoing rejection moot. Notwithstanding the foregoing, Applicants wish to make the following remarks of record.

Applicants respectfully traverse the foregoing rejection on the grounds that one skilled in the art would find the term "about" as used in the pending claims to be clear and definite. Applicants direct the Examiner's attention to MPEP § 2173.05(b) which states that

> [t]he fact that claim language, including terms of degree, may not be precise, does not automatically render the claim indefinite under 35 U.S.C. 112, second paragraph. *Seattle Box Co., v. Industrial Crating & Packing, Inc.*, 731 F.2d 818, 221 USPQ 568 (Fed. Cir. 1984)... The term 'about' used to define the area of the lower end of a mold as between 25 and about 45% of the mold entrance was held to be clear, but flexible. *Ex parte Eastwood*, 163 USPQ 316

> (Bd. App. 1968) Similarly, in *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), the court held that a limitation defining the stretch rate of plastic as 'exceeding about 10% per second' is definite because infringement could clearly be assessed through the use of a stopwatch.

Similarly, the term "about" is used in the pending claims to define an upper limit of flanking nucleotide residues, one that can be clearly assessed through art-known techniques. As such, in accordance with MPEP § 2173.05(b), Applicants submit that one skilled in the art would find the term "about" to be clear and definite as used in the presently pending claims.

Notwithstanding the foregoing and in the interest of expediting allowance of the pending claims, Applicants have amended claims 1 and 40 to delete the term "about," thereby rendering this rejection moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing rejection under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 1, 9-14, 17, 25, 26, 28, 29, 31-33 and 40 Under 35 U.S.C. § 112, First Paragraph (Written Description)

The Examiner has rejected claims 1, 9-14, 17, 25, 26, 28, 29, 31-33 and 40 under 35 U.S.C. § 112, first paragraph as containing subject matter which allegedly was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. In particular, the Examiner is of the opinion that the specification as originally filed does not disclose the limitation "said nucleic acid molecule comprises less than about 5 kb of nucleotide sequence which naturally flank the nucleotide sequence of SEQ ID NO:1."

Applicants traverse the foregoing rejection on the grounds that the specification as originally filed ***explicitly teaches the recited limitation***. Applicants direct the Examiner's attention to page 23, lines 5-8 of the specification where Applicants teach that

> [p]referably, an 'isolated' nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated SMP nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1kb of nucleotide sequences which naturally flank the nucleic acid

molecule in genomic DNA of the cell from which the nucleic acid is derived (e.g., a *C. glutamicum* cell).

As indicated by the foregoing excerpt from Applicants' specification, Applicants were in possession of nucleic acid molecules comprising less than 5 kb of nucleotide sequence which naturally flank the nucleotide sequence of SEQ ID NO:1 at the time of the filing of the present application and, thus, the written description requirement of 35 U.S.C. § 112, first paragraph, has clearly been satisfied. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of the pending claims under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 25, 26, 28, 29 and 31-33 Under 35 U.S.C. § 112, First Paragraph (Enablement)

The Examiner has rejected claims 25, 26, 28, 29 and 31-33 under 35 U.S.C. § 112, first paragraph as not being enabled. In particular, the Examiner is of the opinion that

> [w]hile the specification provides general guidance for transforming isolated *C. glutamicum* host cells with a vector containing the claimed insolated [sic] nucleic acid, the specification does not provide specific guidance, prediction, and working examples for any fine chemical that can be produced by culturing said isolated *C. glutamicum* host cells. Thus, an undue amount of trial and error experimentation must be preformed [sic] to search and screen for any fine chemical that can be produced by culturing the recited cell transformed with the claimed vector comprising the nucleotide sequence of SEQ ID NO:1. Such experimentation is outside the realm of routine experimentation.
>
> In view of the above considerations, the specification does not provide enablement for the claimed methods for making a widely varying fine chemicals such as amino acids, nucleotides, aromatic compounds, vitamins and proteins.

Applicants respectfully traverse the foregoing rejection for the following reasons. Applicants submit that, based on the teachings in Applicants' specification as well as the general knowledge available in the art at the time of the filing of the present application, one of ordinary skill in the art would be able to make and use the claimed invention using only routine experimentation.

As an initial matter, Applicants respectfully submit that the claims have been amended such that they are now directed to methods for modulating the ***production of an amino acid***. The foregoing amendment was made solely in an effort to expedite

examination and allowance of the pending claims and in no way acquiescing to the validity of the Examiner's rejection.

The Specification Enables the Claimed Methods

As provided by M.P.E.P. § 2164.02, the absence of a working example is not sufficient to undermine the enablement of a claimed invention. Indeed, "the specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904." M.P.E.P. § 2164.01(b) also provides that "as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied. In re Fisher, 427 F.2d 833, 839."

In view of the foregoing enablement standards, Applicants respectfully submit that the teachings of the present specification are sufficient to enable an ordinarily skilled artisan to make and use the claimed invention using only routine experimentation. For example, Applicants teach various methods for evaluating the effect of a 6-phosphogluconolactonase molecule on the production of a desired fine chemical such as an amino acid (see page 50, line 6 to page 58, line 22 of the specification). In particular, Examples 4 and 5, at page 51, line 29 to page 53, line 16 of the specification, teach the mutagenesis of host cells with 6-phosphogluconolactonase molecules. Example 6, at page 53, line 18 to page 54, line 4 of the specification, describes techniques for identifying the expression of the 6-phosphogluconolactonase molecules and Example 7, at page 54, line 6 to page 55, line 37 of the specification, describes techniques and culture and media conditions for growing the cells. Example 8, at page 56, lines 1-28 of the specification, describes techniques for analyzing the function of the 6-phosphogluconolactonase molecules. In addition, Example 9, at page 56, line 30 to page 57, line 24 of the specification, describes techniques for analyzing the function of the 6-phosphogluconolactonase molecules on the production of the desired fine chemical. Lastly, Example 10, at page 57, line 26 to page 58, line 22 of the specification, describes techniques for the purification and isolation of the desired product. Applicants submit that one skilled in the art would be able to use such techniques to readily make and use the claimed

invention, *i.e.*, to make and use host cells capable of modulating the production of a fine chemical, such as an amino acid.

Please further argue that one skilled in the art would appreciate that the manipulation of the expression and activity of 6-phosphogluconolactonase nucleic acid and polypeptide molecules would serve to modulate the production of fine chemicals. As was well known in the art at the time of the invention and as taught in the specification, the processing of energy rich carbon molecules is critical to the operation of a cell, for example, as a nutritional source to power basic biochemical pathways. In particular, the pentose phosphate pathway is involved in the processing of glucose to produce reducing equivalents such as NADPH. NADPH, in turn, is critical to the biosynthesis of many amino acids such as glutamate and proline. In addition, the pentose phosphate pathway is involved in the production of intermediates and precursors, for example, pentose and tetrose intermediates which serve as intermediates and precursors in other metabolic pathways. For example, two products of the pentose phosphate pathway, ribose-5-phosphate and erythrose-4-phosphate are important precursors for nucleic acid and amino acid synthesis, respectively (see page 18, line 4 to page 20, line 20 of the specification and Stryer "Biochemistry" (1981) pages 333-354 and 485-536, a copy of which is attached herein as Appendix A). Accordingly, one skilled in the art would appreciate that the enhanced operation of the pentose phosphate pathway would serve to enhance the production of fine chemicals such as nucleotides and amino acids.

The importance of the pentose phosphate pathway in this regard highlights the role that 6-phosphogluconolactonase can play in modulating the production of fine chemicals from a cell. Indeed, 6-phosphogluconolactonase catalyzes the hydrolysis of 6-phosphogluconolactone to gluconate 6-phosphate in the second step of the pentose phosphate pathway (see Stryer "Biochemistry" (1981) pages 333-354). As such, a skilled artisan would appreciate that the specifically defined nucleotide and amino acid sequences of the invention encoding the 6-phosphogluconolactonase enzyme could be utilized to modulate, for example, enhance the production of a desired amino acid. For example, the transformation of a cell to express additional copies of the enzyme or alternatively, the mutation of the enzyme to exhibit enhanced activity, could serve to increase the flux through the pentose phosphate pathway, thereby

increasing the production of products such as ribose-5-phosphate and erythrose-4-phosphate available for the production of fine chemicals such as amino acids.

Post-Filing Date Experimental Evidence Confirms the Teachings in the Specification

The foregoing teachings in the specification and in the art have been confirmed by post-filing date experimental evidence. Specifically, Applicants have demonstrated that a strain of *Corynebacterium glutamicum* in which expression of a gene encoding 6-phosphogluconolactonase was increased resulted in a corresponding ***increase in the production of lysine*** as compared to a control wild type strain of *Corynebacterium glutamicum* (see Appendix B submitted herewith). The enhanced formation of 6-phosphogluconolactonase was also confirmed by the performance of 2-D-gels.

Applicants submit that the techniques utilized in the foregoing experiments to successfully demonstrate the modulation of the production of amino acids, such as lysine, were techniques either taught in the present specification or well known in the art at the time of the filing of the present application. As such, a skilled artisan would be able to use such techniques, without undue experimentation, to make and use host cells with 6-phosphogluconolactonase molecules capable of modulating the production of amino acids.

Accordingly, based on the foregoing teachings in Applicants' specification, the general knowledge in the art at the time of the invention, and the confirmation of such teachings by post-filing date experimental evidence, one skilled in the art would appreciate that the manipulation of the expression and activity of 6-phosphogluconolactonase would serve to modulate the production of fine chemicals such as amino acids.

For each of the foregoing reasons, Applicants submit that the claimed invention is enabled by the teachings in Applicants' specification and, therefore, in compliance with the requirements of 35 U.S.C. §112, first paragraph. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of the pending claims under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 1, 4-6, 9-14, 17, 25, 26, 28, 29, 31-33, 39 and 40 Under 35 U.S.C. § 102(e)

The Examiner has rejected claims 1, 4-6, 9-14, 17, 25, 26, 28, 29, 31-33, 39 and 40 under 35 U.S.C. §102(e) as being anticipated by Dunican *et al.* (USPN 6,797,509) (hereinafter referred to as "Dunican"). Applicants respectfully traverse the foregoing rejection for the following reasons. In the interest of clarity, Applicants address each aspect of this rejection below.

With respect to independent claim 1, and claims depending therefrom, the Examiner states that

> [f]or the reasons stated above in the rejection of the claims under 35 U.S.C. 112, second paragraph, as being indefinite, no patentable weight is given to the phrases 'less than about 5 kb of nucleotide sequences' and 'said nucleic acid molecule comprises less than about 4 kb, 3 kb, 2, kb [sic], 1kb, 0.5kb or 0.1 kb of nucleotide sequences'...

Applicants respectfully submit that, for the reasons provided above, amended claim 1 is clear and definite and satisfies the requirements of 35 U.S.C. § 112, second paragraph. Accordingly, all claim limitations should be given the proper consideration. Applicants further submit that Dunican fails to teach or suggest every limitation of the claimed invention. Specifically, Dunican fails to teach or suggest a nucleic acid molecule comprising SEQ ID NO:1 or encoding a polypeptide comprising SEQ ID NO:2, where the nucleic acid molecule includes less than 5 kb of nucleotide sequences which naturally flank the nucleotide sequence of SEQ ID NO:1 or the nucleotide sequence encoding SEQ ID NO:2, as required by claim 1. Indeed, as demonstrated in Appendix C, enclosed herewith, Dunican discloses a nucleic acid molecule in which the nucleotide sequence shared with the present invention is flanked by over 6 kb of naturally present nucleotide sequences on the 3' region alone. Accordingly, Dunican fails to teach or suggest each and every element of claim 1, and claims dependent therefrom.

With respect to independent claim 4, and claims depending therefrom, the Examiner states that

> [t]he examiner respectfully disagrees with applicants' position that Dunican et al. fail to teach the nucleic acid molecules encoding [polypeptides] having 6-phosphogluconolactonase activity as recited in the amended claims.

> ...Dunican et al. teach a 6995 base pair DNA sequence... that is 100% identical to SEQ ID NO:1 of the claimed invention... The examiner takes the position that in absence of facts to the contrary the DNA taught by Dunican et al. would inherently encode a polypeptide having 6-phosphogluconolactoanase [sic] activity since Dunican et al. teach a 6995 base pair DNA sequence that is 100% identical to SEQ ID NO:1 of the claimed invention.

Applicants also respectfully traverse this rejection for the following reasons. Applicants submit that Dunican fails to teach or suggest nucleic acid molecules comprising SEQ ID NO:1 or encoding a polypeptide comprising SEQ ID NO:2, where the nucleic acid molecule encodes ***only*** a polypeptide having 6-phosphogluconolactonase activity, as required by claim 4. Indeed, if one were to express the nucleic acid molecule of Dunican, as suggested by the Examiner, the nucleic acid molecule would encode ***at least two polypeptides***, one of which would have transaldolase activity. Accordingly, Dunican fails to teach or suggest a nucleic acid molecule which encodes ***only*** a polypeptide having 6-phosphogluconolactonase activity and, thereby, fails to teach or suggest each and every element of claim 4, and claims depending therefrom.

In view of all of the foregoing, Applicants respectfully request reconsideration and withdrawal of the rejection of the pending claims under 35 U.S.C. § 102(e).

CONCLUSION

In view of the foregoing remarks, reconsideration of the rejections and allowance of all pending claims is respectfully requested. If there are any remaining issues or if the Examiner believes that a telephone conversation with Applicants' Attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at (617) 227-7400.

Dated: October 16, 2006

Respectfully submitted,

By _____

Maria Laccotripe Zacharakis, Ph.D., J.D.
Registration No.: 56,266
Attorney for Applicants
LAHIVE & COCKFIELD, LLP
28 State Street
Boston, Massachusetts 02109

APPENDIX

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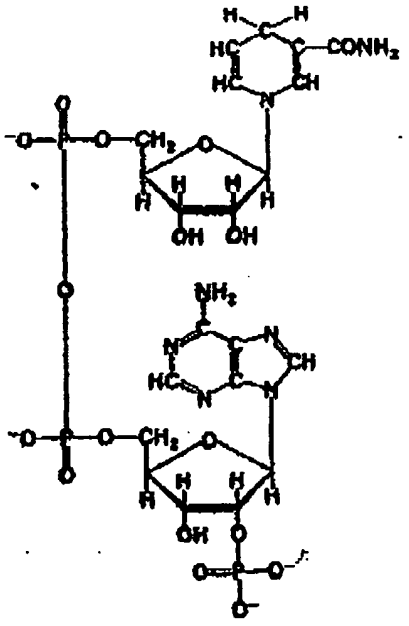
CHAPTER 15

PENTOSE PHOSPHATE PATHWAY AND GLUCONEOGENESIS

The preceding chapters on glycolysis, the citric acid cycle, and oxidative phosphorylation were primarily concerned with the generation of ATP, starting with glucose as a fuel. We now turn to the generation of a different type of metabolic energy—reducing power. Some of the electrons and hydrogen atoms of fuel molecules must be conserved for biosynthetic purposes rather than transferred to O_2 to generate ATP. *The currency of readily available reducing power in cells is NADPH.* The phosphoryl group on C-2 of one of the ribose units of NADPH distinguishes it from NADH. As mentioned previously (p. 246), there is a *fundamental distinction between NADPH and NADH in most biochemical reactions. NADH is oxidized by the respiratory chain to generate ATP, whereas NADPH serves as a hydrogen and electron donor in reductive biosyntheses.* This chapter also deals with the synthesis of glucose from noncarbohydrate precursors, a process called *gluconeogenesis.*



Reduced nicotinamide adenine dinucleotide phosphate (NADPH)

THE PENTOSE PHOSPHATE PATHWAY GENERATES NADPH AND SYNTHESIZES FIVE-CARBON SUGARS

In the pentose phosphate pathway, NADPH is generated as glucose 6-phosphate is oxidized to ribose 5-phosphate. This five-carbon

sugar and its derivatives are components of such important biomolecules as ATP, CoA, NAD^+, FAD, RNA, and DNA.

$$\text{Glucose 6-phosphate} + 2\,NADP^+ + H_2O \longrightarrow \text{ribose 5-phosphate} + 2\,NADPH + 2\,H^+ + CO_2$$

The pentose phosphate pathway also catalyzes the interconversion of three-, four-, five-, six-, and seven-carbon sugars in a series of nonoxidative reactions. All of these reactions occur in the cytosol. In plants, part of the pentose phosphate pathway also participates in the formation of hexoses from CO_2 in photosynthesis (Chapter 19).

The pentose phosphate pathway is sometimes called *the pentose shunt, the hexose monophosphate pathway,* or *the phosphogluconate oxidative pathway*. The discovery of glucose 6-phosphate dehydrogenase, the first enzyme in the pathway, by Otto Warburg in 1931 led to its complete elucidation by Fritz Lipmann, Frank Dickens, Bernard Horecker, and Efraim Racker.

TWO NADPH ARE GENERATED IN THE CONVERSION OF GLUCOSE 6-PHOSPHATE INTO RIBULOSE 5-PHOSPHATE

The pentose phosphate pathway starts with the dehydrogenation of glucose 6-phosphate at C-1, a reaction catalyzed by *glucose 6-phosphate dehydrogenase* (Figure 15-1). This enzyme is highly specific for

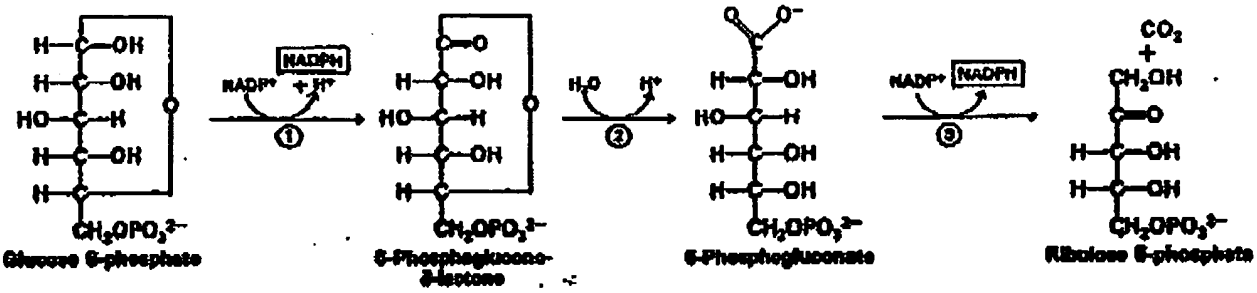
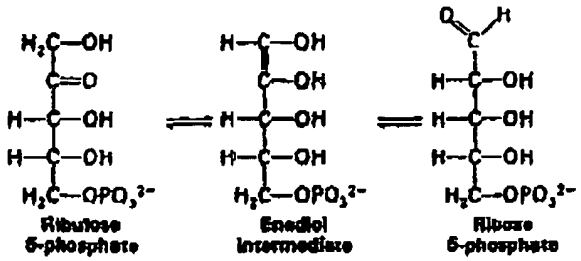


Figure 15-1
Oxidative branch of the pentose phosphate pathway. These three reactions are catalyzed by glucose 6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase.

$NADP^+$; the K_M for NAD^+ is about a thousand times as great as that for $NADP^+$. The product is *6-phosphoglucono-δ-lactone*, which is an intramolecular ester between the C-1 carboxyl group and the C-5 hydroxyl group. The next step is the hydrolysis of 6-phosphoglucono-δ-lactone by a specific *lactonase* to give *6-phosphogluconate*. This six-carbon sugar is then oxidatively decarboxylated by *6-phosphogluconate dehydrogenase* to yield *ribulose 5-phosphate*. $NADP^+$ is again the electron acceptor.

RIBULOSE 5-PHOSPHATE IS ISOMERIZED TO RIBOSE 5-PHOSPHATE THROUGH AN ENEDIOL INTERMEDIATE

The final step in the synthesis of ribose 5-phosphate is the isomerization of ribulose 5-phosphate by *phosphopentose isomerase*.



This reaction is similar to the glucose 6-phosphate ⇌ fructose 6-phosphate and to the dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate reactions in glycolysis. *These three ketose-aldose isomerizations proceed through an enediol intermediate.*

THE PENTOSE PHOSPHATE PATHWAY AND GLYCOLYSIS ARE LINKED BY TRANSKETOLASE AND TRANSALDOLASE

The preceding reactions yield two NADPH and one ribose 5-phosphate for each glucose 6-phosphate oxidized. However, many cells need much more NADPH for reductive biosyntheses than ribose 5-phosphate for incorporation into nucleotides and nucleic acids. Under these conditions, ribose 5-phosphate is converted into glyceraldehyde 3-phosphate and fructose 6-phosphate by *transketolase* and *transaldolase. These enzymes create a reversible link between the pentose phosphate pathway and glycolysis* by catalyzing these three reactions:

$$C_5 + C_5 \xrightleftharpoons{\text{transketolase}} C_3 + C_7$$

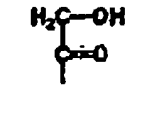
$$C_7 + C_3 \xrightleftharpoons{\text{transaldolase}} C_4 + C_6$$

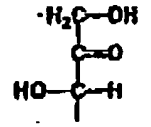
$$C_5 + C_4 \xrightleftharpoons{\text{transketolase}} C_3 + C_6$$

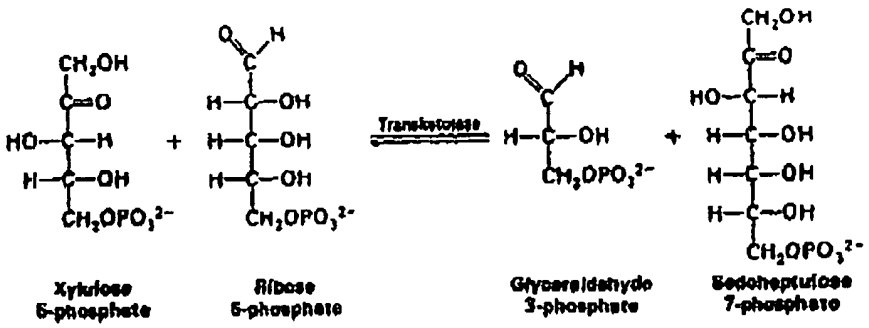
The sum of these reactions is the *formation of two hexoses and one triose from three pentoses.*

The essence of these reactions is that *transketolase transfers a two-carbon unit, whereas transaldolase transfers a three-carbon unit.* The sugar that donates the two- or three-carbon unit is always a ketose, whereas the acceptor is always an aldose.

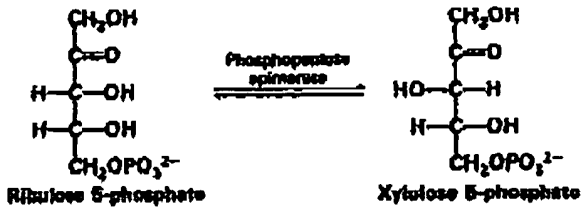
The first of the three reactions linking the pentose phosphate pathway and glycolysis is the formation of *glyceraldehyde 3-phosphate* and *sedoheptulose 7-phosphate* from two pentoses.


Transferred by transketolase

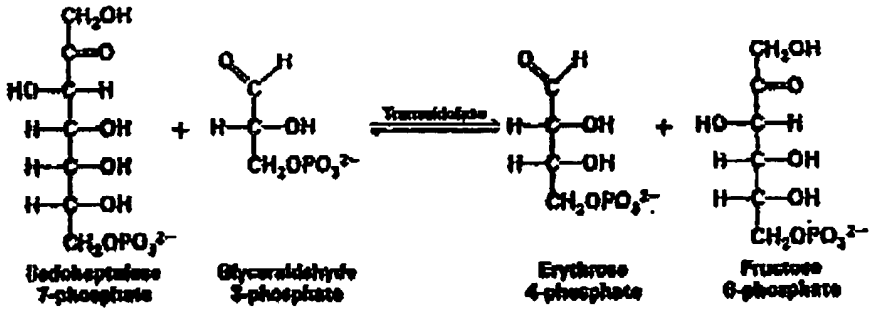

Transferred by transaldolase



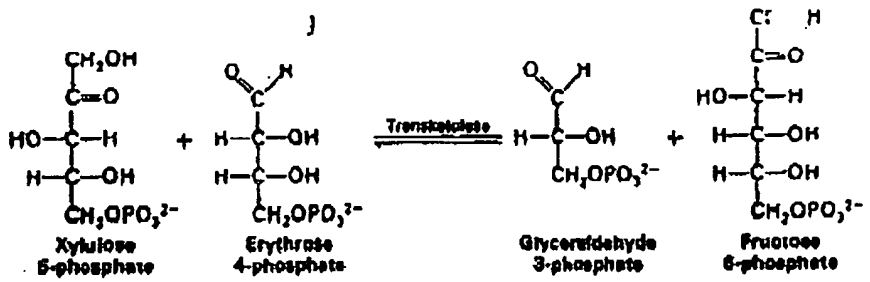
The donor of the two-carbon unit in this reaction is xylulose 5-phosphate, which is an epimer of ribulose 5-phosphate. A ketose is a substrate of transketolase only if its hydroxyl group at C-3 has the configuration of xylulose rather than ribulose. Ribulose 5-phosphate is converted into the appropriate epimer for the transketolase reaction by *phosphopentose epimerase*.



Glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate then react to form *fructose 6-phosphate* and *erythrose 4-phosphate*. This synthesis of a four-carbon sugar and a six-carbon sugar is catalyzed by *transaldolase*.



In the third reaction, transketolase catalyzes the synthesis of *fructose 6-phosphate* and *glyceraldehyde 3-phosphate* from erythrose 4-phosphate and xylulose 5-phosphate.



The sum of these reactions is

2 Xylulose 5-phosphate + ribose 5-phosphate ⇌
2 fructose 6-phosphate + glyceraldehyde 3-phosphate

Xylulose 5-phosphate can be formed from ribose 5-phosphate by the sequential action of phosphopentose isomerase and phosphopentose epimerase, and so the net reaction starting from ribose 5-phosphate is

3 Ribose 5-phosphate ⇌
2 fructose 6-phosphate + glyceraldehyde 3-phosphate

Thus, excess ribose 5-phosphate formed by the pentose phosphate pathway can be quantitatively converted into glycolytic intermediates.

Table 16-1
Pentose phosphate pathway

Reaction	*Enzyme*
OXIDATIVE BRANCH	
Glucose 6-phosphate + $NADP^+$ ⇌ 6-phosphoglucono-δ-lactone + NADPH + H^+	Glucose 6-phosphate dehydrogenase
6-Phosphoglucono-δ-lactone + H_2O → 6-phosphogluconate + H^+	Lactonase
6-Phosphogluconate + $NADP^+$ → ribulose 5-phosphate + CO_2 + NADPH	6-Phosphogluconate dehydrogenase
NONOXIDATIVE BRANCH	
Ribulose 5-phosphate ⇌ ribose 5-phosphate	Phosphopentose isomerase
Ribulose 5-phosphate ⇌ xylulose 5-phosphate	Phosphopentose epimerase
Xylulose 5-phosphate + ribose 5-phosphate ⇌ sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate	Transketolase
Sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate ⇌ fructose 6-phosphate + erythrose 4-phosphate	Transaldolase
Xylulose 5-phosphate + erythrose 4-phosphate ⇌ fructose 6-phosphate + glyceraldehyde 3-phosphate	Transketolase

THE RATE OF THE PENTOSE PHOSPHATE PATHWAY IS CONTROLLED BY THE LEVEL OF $NADP^+$

The first reaction in the oxidative branch of the pentose phosphate pathway, the dehydrogenation of glucose 6-phosphate, is essentially irreversible. In fact, this reaction is rate-limiting under physiologi-

cal conditions and serves as the control site. The most important regulatory factor is the level of $NADP^+$, the electron acceptor in the oxidation of glucose 6-phosphate to 6-phosphogluconolactone. Also, NADPH competes with $NADP^+$ in binding to the enzyme, and ATP competes with glucose 6-phosphate. The ratio of $NADP^+$ to NADPH in the cytosol from the liver of a well-fed rat is about 0.014, several orders of magnitude lower than the ratio of NAD^+ to NADH, which is 700 under the same conditions. The marked effect of the $NADP^+$ level on the rate of the oxidative branch ensures that NADPH generation is tightly coupled to its utilization in reductive biosyntheses. The control of the nonoxidative branch of the pentose phosphate pathway has not yet been defined.

THE FLOW OF GLUCOSE 6-PHOSPHATE DEPENDS ON THE NEED FOR NADPH, RIBOSE 5-PHOSPHATE, AND ATP

Let us follow the fate of glucose 6-phosphate in four different situations:

1. *Much more ribose 5-phosphate than NADPH is required.* Most of the glucose 6-phosphate is converted into fructose 6-phosphate and glyceraldehyde 3-phosphate by the glycolytic pathway. Transaldolase and transketolase then convert two molecules of fructose 6-phosphate and one molecule of glyceraldehyde 3-phosphate into three molecules of ribose 5-phosphate by a reversal of the reactions described earlier. The stoichiometry of this mode (Figure 15-2A) is

$$5 \text{ Glucose 6-phosphate} + 5 \text{ ATP} \longrightarrow 6 \text{ ribose 5-phosphate} + 5 \text{ ADP} + 5 \text{ H}^+$$

2. *The needs for NADPH and ribose 5-phosphate are balanced.* The predominant reaction under these conditions is the formation of two NADPH and one ribose 5-phosphate from glucose 6-phosphate by the oxidative branch of the pentose phosphate pathway. The stoichiometry of this mode (Figure 15-2B) is

$$\text{Glucose 6-phosphate} + 2\,\text{NADP}^+ + \text{H}_2\text{O} \longrightarrow \text{ribose 5-phosphate} + 2\,\text{NADPH} + 2\,\text{H}^+ + \text{CO}_2$$

3. *Much more NADPH than ribose 5-phosphate is required; glucose 6-phosphate is completely oxidized to CO_2.* Three groups of reactions are active in this situation. First, two NADPH and one ribose 5-phosphate are formed by the oxidative branch of the pentose phosphate pathway. Then, ribose 5-phosphate is converted into fructose 6-phosphate and glyceraldehyde 3-phosphate by transketolase and transaldolase. Finally, glucose 6-phosphate is resynthesized from fructose 6-phosphate and glyceraldehyde 3-phosphate by the gluconeogenic pathway (discussed later in this chapter). The stoichiometries of these three sets of reactions (Figure 15-2C) are

$$6 \text{ Glucose 6-phosphate} + 12\,\text{NADP}^+ + 6\,\text{H}_2\text{O} \longrightarrow 6 \text{ ribose 5-phosphate} + 12\,\text{NADPH} + 12\,\text{H}^+ + 6\,\text{CO}_2$$

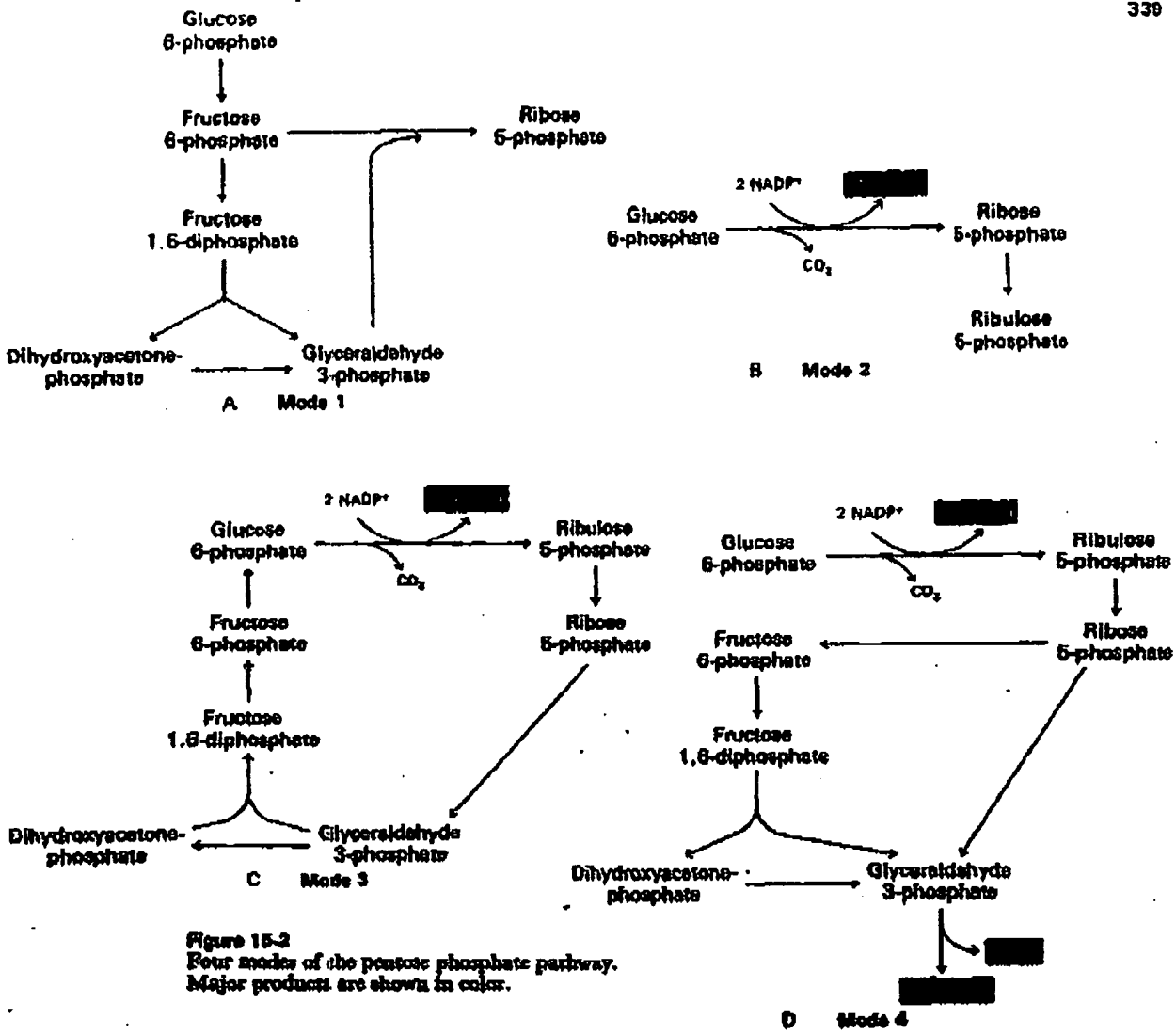


Figure 15-2
Four modes of the pentose phosphate pathway. Major products are shown in color.

$$6 \text{ Ribose 5-phosphate} \longrightarrow 4 \text{ fructose 6-phosphate} + 2 \text{ glyceraldehyde 3-phosphate}$$

$$4 \text{ Fructose 6-phosphate} + 2 \text{ glyceraldehyde 3-phosphate} + H_2O \longrightarrow 5 \text{ glucose 6-phosphate} + P_i$$

The sum of these reactions is

$$\text{Glucose 6-phosphate} + 12\,NADP^+ + 7\,H_2O \longrightarrow 6\,CO_2 + 12\,NADPH + 12\,H^+ + P_i$$

Thus, the equivalent of a glucose 6-phosphate can be completely oxidized to CO_2 with the concomitant generation of NADPH. The essence of these reactions is that *the ribose 5-phosphate produced by the pentose phosphate pathway is recycled into glucose 6-phosphate* by transketolase, transaldolase, and some of the enzymes of the gluconeogenic pathway.

4. *Much more NADPH than ribose 5-phosphate is required; glucose 6-phosphate is converted into pyruvate.* Alternatively, ribose 5-phosphate formed by the oxidative branch of the pentose phosphate pathway can be converted into pyruvate (Figure 15-2D). Fructose 6-phosphate and glyceraldehyde 3-phosphate derived from ribose 5-phosphate proceed down the glycolytic pathway rather than reverting to glucose 6-phosphate. In this mode, *ATP and NADPH are concomitantly generated, and five of the six carbons of glucose 6-phosphate emerge in pyruvate:*

$$3\ \text{Glucose 6-phosphate} + 6\ NADP^+ + 5\ NAD^+ + 5\ P_i + 8\ ADP \longrightarrow 5\ \text{pyruvate} + 3\ CO_2 + 6\ NADPH + 5\ NADH + 8\ ATP + 2\ H_2O + 8\ H^+$$

Pyruvate formed by these reactions can be oxidized to generate more ATP or it can be used as a building block in a variety of biosyntheses.

Figure 15-3
Light micrograph of adipose tissue.

THE PENTOSE PHOSPHATE PATHWAY IS MUCH MORE ACTIVE IN ADIPOSE TISSUE THAN IN MUSCLE

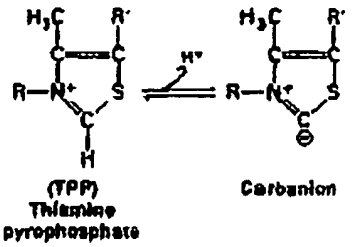
Radioactive-labeling experiments can provide estimates of how much glucose 6-phosphate is metabolized by the pentose phosphate pathway and how much is metabolized by the combined action of glycolysis and the citric acid cycle. One aliquot of a tissue homogenate is incubated with glucose labeled with ^{14}C at C-1, and another with glucose labeled with ^{14}C at C-6. The radioactivity of the CO_2 produced by the two samples is then compared. The rationale of this experiment is that only C-1 is decarboxylated by the pentose phosphate pathway, whereas C-1 and C-6 are decarboxylated to the same extent when glucose is metabolized by the glycolytic pathway, the pyruvate dehydrogenase complex, and the citric acid cycle. The reason for the equivalence of C-1 and C-6 in the latter set of reactions is that glyceraldehyde 3-phosphate and dihydroxyacetone phosphate are rapidly interconverted by triose phosphate isomerase.

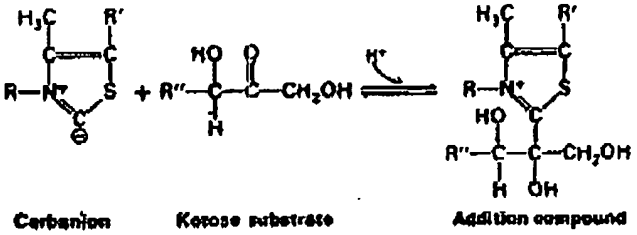
This experimental approach has shown that *the activity of the pentose phosphate pathway is very low in skeletal muscle, whereas it is very high in adipose tissue.* These findings support the idea that a major role of the pentose phosphate pathway is to generate NADPH for reductive biosyntheses. Large amounts of NADPH are consumed by adipose tissue in the reductive synthesis of fatty acids from acetyl CoA (see Chapter 17).

TPP, THE PROSTHETIC GROUP OF TRANSKETOLASE, TRANSFERS ACTIVATED ALDEHYDES

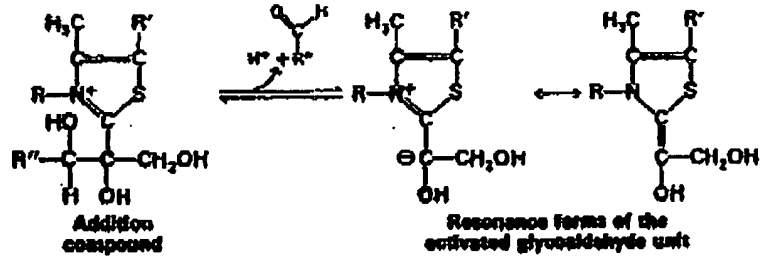
Transketolase contains a tightly bound thiamine pyrophosphate (TPP) as its prosthetic group. This prosthetic group has been en-

countered before in the decarboxylation of pyruvate by the pyruvate dehydrogenase complex. The mechanism of catalysis of transketolase is similar in that an *activated aldehyde unit is transferred to an acceptor*. The acceptor in the transketolase reaction is an aldose, whereas in the pyruvate dehydrogenase reaction it is lipoamide. In both reactions, the site of addition of the keto substrate is the *thiazole ring* of the prosthetic group. The C-2 carbon atom is highly acidic and readily ionizes to give a *carbanion*. This carbanion adds to the carbonyl group of the ketose substrate (e.g., xylulose 5-phosphate, fructose 6-phosphate, and sedoheptulose 7-phosphate).

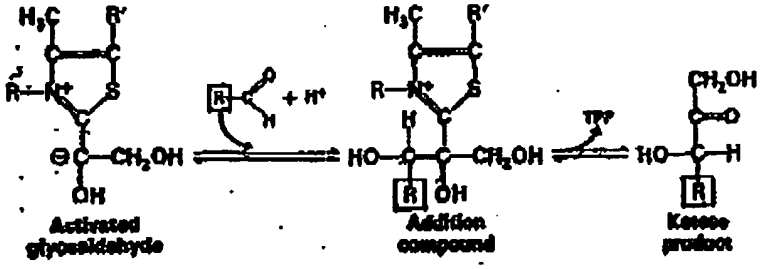




This addition compound loses its R—CHOH moiety to yield a negatively charged, *activated glycoaldehyde* unit. The positively charged nitrogen in the thiazole ring acts as an electron sink to promote the development of a negative charge on the activated intermediate.



The carbonyl group of a suitable aldehyde acceptor then condenses with the activated glycoaldehyde unit to form a new ketose, which is released from the enzyme.

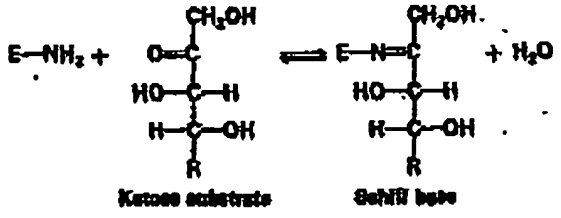


TRANSKETOLASE DEFECTIVE IN TPP BINDING CAN CAUSE A NEUROPSYCHIATRIC DISORDER

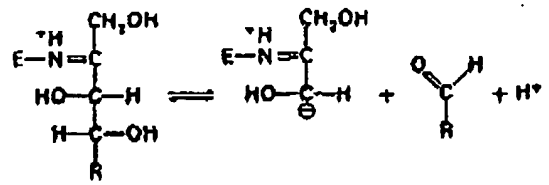
The *Wernicke-Korsakoff syndrome*, a striking neuropsychiatric disorder, is caused by a lack of thiamine in the diet of susceptible persons. This disease is characterized by paralysis of eye movements, abnormal stance and gait, and markedly deranged mental function. In particular, a patient with this syndrome is disoriented and has a severely impaired memory. It has been known for some time that only a small minority of alcoholics and other chronically malnourished persons develop this disorder. Also, its incidence is much higher among Europeans than among non-Europeans on thiamine-deficient diets. These observations suggest that genetic factors may be important determinants of whether a thiamine-deficient person develops the Wernicke-Korsakoff syndrome. Recent studies of transketolase from cultured fibroblasts show that this is in fact the case. *Transketolase from patients with the Wernicke-Korsakoff syndrome binds thiamine pyrophosphate tenfold less avidly than does the enzyme from normal persons.* The other two thiamine-dependent enzymes, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, are normal in this disorder. The abnormality in transketolase becomes clinically evident only when the level of thiamine pyrophosphate is too low to saturate the enzyme. This is a clear-cut example of the interplay between genetic and environmental factors in the production of disease. The Wernicke-Korsakoff syndrome also provides a vivid demonstration of how a reduction in the activity of a single enzyme can have profound neurologic and behavioral consequences.

ACTIVATED DIHYDROXYACETONE IS CARRIED BY TRANSALDOLASE AS A SCHIFF BASE

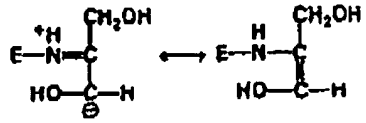
Transaldolase transfers a three-carbon *dihydroxyacetone* unit from a ketose donor to an aldose acceptor. Transaldolase, in contrast with transketolase, does not contain a prosthetic group. Rather, *a Schiff base is formed between the carbonyl group of the ketose substrate and the ε-amino group of a lysine residue at the active site of the enzyme.* This kind of covalent enzyme-substrate (ES) intermediate is like that formed in fructose diphosphate aldolase in the glycolytic pathway.



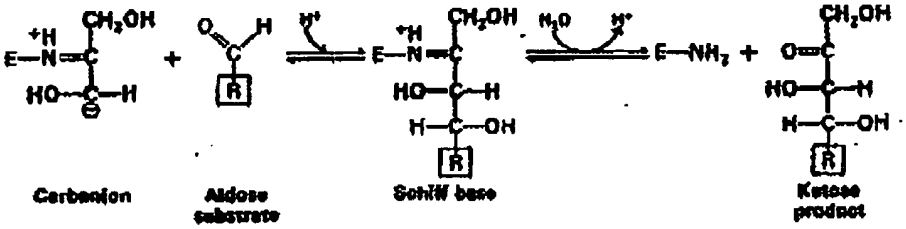
The Schiff base becomes protonated, the bond between C-3 and C-4 is split, and an aldose is released.



The negative charge on the dihydroxyacetone moiety is stabilized by resonance. The positively charged nitrogen atom of the Schiff base acts as an electron sink. This nitrogen atom plays the same role in transaldolase as does the thiazole-ring nitrogen in transketolase.



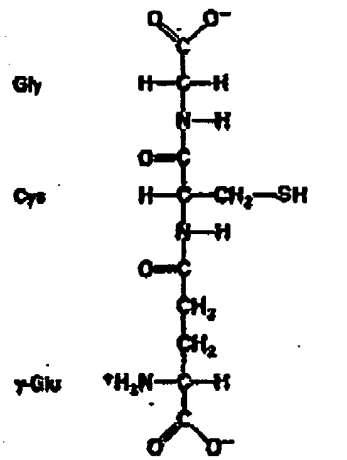
The Schiff base between dihydroxyacetone and transaldolase is stable until a suitable aldose becomes bound. The carbanion of the dihydroxyacetone moiety then reacts with the carbonyl group of the aldose. The ketose product is released by hydrolysis of the Schiff base.

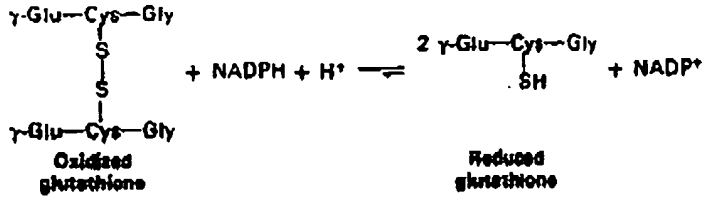


GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY CAUSES A DRUG-INDUCED HEMOLYTIC ANEMIA

An antimalarial drug, pamaquine, was introduced in 1926. Most patients tolerated the drug well, but a few developed severe symptoms within a few days after therapy was started. The urine turned black, jaundice developed, and the hemoglobin content of the blood dropped sharply. In some cases, massive destruction of red blood cells caused death.

The basis of this *drug-induced hemolytic anemia* was elucidated in 1956. The primary defect is a *deficiency in glucose 6-phosphate dehydrogenase in red cells*. The pentose phosphate pathway is the only source of NADPH in red cells, and so the production of NADPH is diminished in glucose 6-phosphate dehydrogenase deficiency. The major role of NADPH in red cells is to reduce the disulfide form of *glutathione* to the sulfhydryl form. This reaction is catalyzed by *glutathione reductase*.





The reduced form of glutathione, a tripeptide with a free sulfhydryl group, serves as a *sulfhydryl buffer* that maintains the cysteine residues of hemoglobin and other red-cell proteins in the reduced state. The ratio of the reduced form of glutathione (GSH) to the oxidized form (GSSG) is normally about 500. The reduced form also plays a role in detoxification by reacting with hydrogen peroxide and organic peroxides.

$$2\,GSH + R{-}O{-}OH \longrightarrow GSSG + H_2O + ROH$$

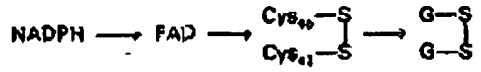
Reduced glutathione appears to be essential for maintaining normal red-cell structure and for keeping hemoglobin in the ferrous state. Cells with a lowered level of reduced glutathione are more susceptible to hemolysis for reasons that are not yet understood. Drugs such as pamaquine may distort the surface of red cells in the absence of reduced glutathione, which would make them more liable to destruction and removal by the spleen. These drugs also increase the rate of formation of toxic peroxides, which are normally eliminated by reaction with reduced glutathione.

Glucose 6-phosphate dehydrogenase deficiency is not a rare disease. It is inherited as a sex-linked trait. Female heterozygotes have two populations of red cells: one has normal enzymatic activity, whereas the other is deficient in glucose 6-phosphate dehydrogenase. The glucose 6-phosphate dehydrogenase in most other organs is specified by a different gene. The incidence of the most common (type A) deficiency of glucose 6-phosphate dehydrogenase, characterized by a tenfold reduction in enzymatic activity in red cells, is 11% among black Americans. This high frequency suggests that the deficiency may be advantageous under certain environmental conditions. Indeed, *glucose 6-phosphate dehydrogenase deficiency in red cells seems to protect a person from falciparum malaria*, because the parasites that cause this disease require the pentose phosphate pathway and reduced glutathione for optimal growth. Thus, glucose 6-phosphate dehydrogenase deficiency and sickle-cell trait are parallel mechanisms of protection against malaria, which accounts for their high gene frequencies in malaria-infested regions of the world.

The occurrence of glucose 6-phosphate dehydrogenase deficiency clearly demonstrates that *atypical reactions to drugs may have a genetic basis*. This inherited enzymatic deficiency is relatively benign until certain drugs are administered. We again see here the interplay of heredity and environment in the production of disease. Galactosemia, hereditary fructose intolerance, phenylketonuria, and succinylcholine sensitivity also illustrate this interaction in a striking way.

GLUTATHIONE REDUCTASE TRANSFERS ELECTRONS FROM NADPH TO OXIDIZED GLUTATHIONE BY WAY OF FAD

The regeneration of reduced glutathionine is catalyzed by glutathione reductase, a dimer of 50-kdal subunits. The electrons from NADPH are not directly transferred to the disulfide bond in oxidized glutathione. Rather, they are transferred from NADPH to a tightly bound flavin adenine dinucleotide (FAD), then to a disulfide bridge between two cysteine residues in the subunit, and finally to oxidized glutathione.



Each subunit consists of three structural domains: an FAD-binding domain, an NADPH-binding domain, and an interface domain (Figure 15-4). The FAD domain and $NADP^+$ domain resemble

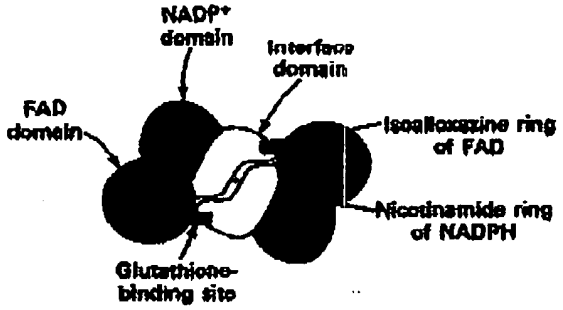


Figure 15-4
Schematic diagram of the domain structure of glutathione reductase. Each subunit in this dimeric enzyme consists of an $NADP^+$ domain, an FAD domain, and an interface domain. Glutathione is bound to the FAD domain of one subunit and the interface domain of another. [After G. E. Schulz, R. H. Schirmer, W. Sachsenheimer, and E. F. Pai, *Nature* 273(1978):123.]

each other and are similar to nucleotide-binding domains in other dehydrogenases. FAD and $NADP^+$ are bound in an extended form, with their isoalloxazine and nicotinamide rings next to each other (Figure 15-4). It is interesting to note that the binding site for oxidized glutathione is formed by the FAD domain of one subunit and the interface domain of the other subunit.

GLUCOSE CAN BE SYNTHESIZED FROM NONCARBOHYDRATE PRECURSORS

We now turn to the *synthesis of glucose from noncarbohydrate precursors*, a process called *gluconeogenesis*. This metabolic pathway is very important because certain tissues, such as the brain, are highly dependent on glucose as the primary fuel. The daily glucose requirement of the brain in a typical adult is about 120 g, which accounts for most of the 160 g of glucose needed by the whole body. The amount of glucose present in body fluids is about 20 g, and that readily available from glycogen, a storage form of glucose (p. 357), is approximately 190 g. Thus, the direct glucose reserves are sufficient to meet

the needs for glucose for about a day. In a longer period of starvation, glucose must be formed from noncarbohydrate sources for survival. Gluconeogenesis is also important during periods of intense exercise.

The major noncarbohydrate precursors of glucose are *lactate, amino acids,* and *glycerol.* Lactate is formed by active skeletal muscle when the rate of glycolysis exceeds the metabolic rate of the citric acid cycle and the respiratory chain (p. 269). Amino acids are derived from proteins in the diet and, during starvation, from the breakdown of proteins in skeletal muscle (p. 553). The hydrolysis of triacylglycerols (p. 386) in fat cells yields glycerol and fatty acids. Glycerol is a precursor of glucose, but fatty acids cannot be converted into glucose in animals, for reasons that will be discussed later (p. 394). The *gluconeogenic pathway converts pyruvate into glucose.* The principal points of entry are pyruvate, oxaloacetate, and dihydroxyacetone phosphate (Figure 15-5).

The major site of gluconeogenesis is the *liver.* Gluconeogenesis also occurs in the cortex of the *kidney,* but the total amount of glucose formed there is about one-tenth of that formed in the liver

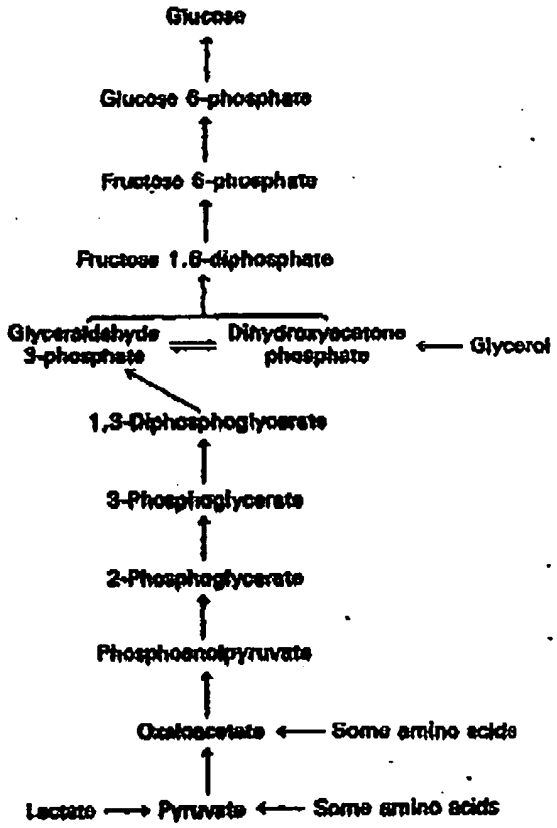


Figure 15-5
Pathway of gluconeogenesis. The distinctive reactions of this pathway are denoted by red arrows. The other reactions are common to glycolysis. The enzymes of gluconeogenesis are located in the cytosol, except for pyruvate carboxylase (in mitochondria), and glucose 6-phosphatase (attached to the endoplasmic reticulum). The entry points for lactate, glycerol, and amino acids are shown.

because of the kidney's smaller mass. Very little gluconeogenesis takes place in the brain, skeletal muscle, or heart muscle. Rather, *gluconeogenesis in the liver and kidney helps to maintain the glucose level in the blood so that brain and muscle can extract sufficient glucose from it to meet their metabolic demands.*

GLUCONEOGENESIS IS NOT A REVERSAL OF GLYCOLYSIS

In glycolysis, glucose is converted into pyruvate; in gluconeogenesis, pyruvate is converted into glucose. However, gluconeogenesis is not a reversal of glycolysis. A different pathway is required because the thermodynamic equilibrium of glycolysis lies far on the side of pyruvate formation. The actual ΔG for the formation of pyruvate from glucose is about -20 kcal/mol under typical cellular conditions (p. 267). Most of the decrease in free energy in glycolysis takes place in the three essentially irreversible steps catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose 6-phosphate} + \text{ADP}$$

$$\text{Fructose 6-phosphate} + \text{ATP} \xrightarrow{\text{phosphofructokinase}} \text{fructose 1,6-diphosphate} + \text{ADP}$$

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow{\text{pyruvate kinase}} \text{pyruvate} + \text{ATP}$$

In gluconeogenesis, these virtually irreversible reactions of glycolysis are bypassed by the following new steps:

1. *Phosphoenolpyruvate is formed from pyruvate by way of oxaloacetate.* First, pyruvate is carboxylated to oxaloacetate at the expense of an ATP. Then, oxaloacetate is decarboxylated and phosphorylated to yield phosphoenolpyruvate, at the expense of a second high-energy phosphate bond.

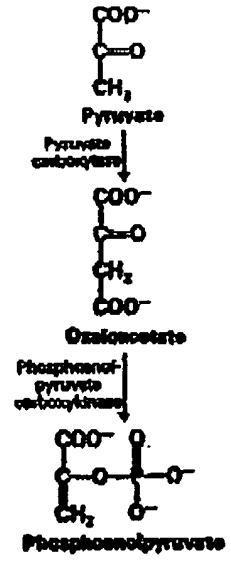
$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \rightleftharpoons \text{oxaloacetate} + \text{ADP} + P_i + 2\,H^+$$

$$\text{Oxaloacetate} + \text{GTP} \rightleftharpoons \text{phosphoenolpyruvate} + \text{GDP} + CO_2$$

The first reaction is catalyzed by *pyruvate carboxylase*, and the second by *phosphoenolpyruvate carboxykinase*. The sum of these reactions is

$$\text{Pyruvate} + \text{ATP} + \text{GTP} + H_2O \rightleftharpoons \text{phosphoenolpyruvate} + \text{ADP} + \text{GDP} + P_i + 2\,H^+$$

This pathway for the formation of phosphoenolpyruvate from pyruvate is thermodynamically feasible, because $\Delta G^{\circ\prime}$ is $+0.2$ kcal/mol in contrast with $+7.5$ kcal/mol for the reaction catalyzed by pyruvate kinase. This much more favorable $\Delta G^{\circ\prime}$ results from the input of an additional high-energy phosphate bond.



2. *Fructose 6-phosphate is formed from fructose 1,6-diphosphate by hydrolysis of the phosphate ester at C-1.* Fructose 1,6-diphosphatase catalyzes this exergonic hydrolysis.

$$\text{Fructose 1,6-diphosphate} + H_2O \longrightarrow \text{fructose 6-phosphate} + P_i$$

3. *Glucose is formed by hydrolysis of glucose 6-phosphate,* a reaction catalyzed by glucose 6-phosphatase.

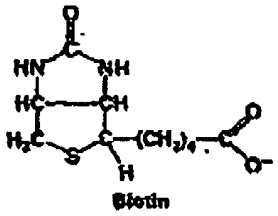
$$\text{Glucose 6-phosphate} + H_2O \longrightarrow \text{glucose} + P_i$$

Glucose 6-phosphatase is bound to the endoplasmic reticulum and acts on substrate located in the cytosol. This enzyme is not present in brain and muscle; hence, glucose does not leave these organs.

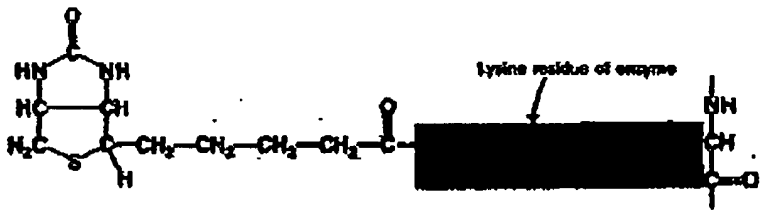
Table 15-2
Enzymatic differences between glycolysis and gluconeogenesis

Glycolysis	*Gluconeogenesis*
Hexokinase	Glucose 6-phosphatase
Phosphofructokinase	Fructose 1,6-diphosphatase
Pyruvate kinase	Pyruvate carboxylase Phosphoenolpyruvate carboxykinase

BIOTIN IS A MOBILE CARRIER OF ACTIVATED CO_2


Biotin

The finding that mitochondria can form oxaloacetate from pyruvate led to the discovery of pyruvate carboxylase by Merton Utter in 1960. This enzyme is of especial interest because of its catalytic and allosteric properties. Pyruvate carboxylase contains a covalently attached prosthetic group, *biotin*, which serves as a *carrier of activated* CO_2. The carboxyl terminus of biotin is linked to the ϵ-amino group of a specified lysine residue by an amide bond.



Note that biotin is attached to pyruvate carboxylase by a *long, flexible chain* like that of lipoamide in the pyruvate dehydrogenase complex.

The carboxylation of pyruvate occurs in two stages:

$$\text{Biotin-enzyme} + ATP + HCO_3^- \xrightleftharpoons[\text{acetyl CoA}]{Mg^{2+}} CO_2 \sim \text{biotin-enzyme} + ADP + P_i$$

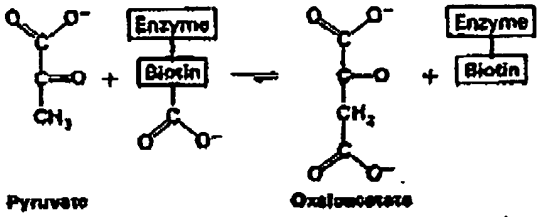
$$CO_2 \sim \text{biotin-enzyme} + \text{pyruvate} \xrightleftharpoons{Mn^{2+}} \text{biotin-enzyme} + \text{oxaloacetate}$$

The carboxyl group in the carboxybiotin-enzyme intermediate is bonded to the N-1 nitrogen atom of the biotin ring. The carboxyl group in this carboxybiotin intermediate is *activated*. The $\Delta G^{\circ\prime}$ for its cleavage

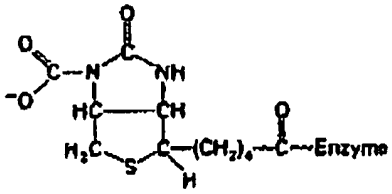
$$CO_2 \sim \text{biotin-enzyme} + H^+ \longrightarrow CO_2 + \text{biotin-enzyme}$$

is −4.7 kcal/mol, which enables carboxybiotin to transfer CO_2 to acceptors without the input of additional free energy.

The activated carboxyl group is then transferred from carboxybiotin to pyruvate to form oxaloacetate. The long, flexible link between biotin and the enzyme enables this prosthetic group to rotate from one active site of the enzyme (the ATP-bicarbonate site) to the other (the pyruvate site).



Pyruvate Oxaloacetate



Carboxybiotin-enzyme intermediate

PYRUVATE CARBOXYLASE IS ACTIVATED BY ACETYL CoA

The activity of pyruvate carboxylase depends on the presence of acetyl CoA. *Biotin is not carboxylated unless acetyl CoA (or a closely related acyl CoA) is bound to the enzyme.* The second partial reaction is not affected by acetyl CoA. The allosteric activation of pyruvate carboxylase by acetyl CoA is an important physiological control mechanism. Oxaloacetate, the product of the pyruvate carboxylase reaction, is both a stoichiometric intermediate in gluconeogenesis and a catalytic intermediate in the citric acid cycle. *A high level of acetyl CoA signals the need for more oxaloacetate.* If there is a surplus of ATP, oxaloacetate will be consumed in gluconeogenesis. If there is a deficiency of ATP, oxaloacetate will enter the citric acid cycle upon condensing with acetyl CoA.

Thus, not only is pyruvate carboxylase important in gluconeogenesis, but it also plays a *critical role in maintaining the level of citric acid cycle intermediates.* These intermediates need to be replenished because they are consumed in some biosynthetic reactions, such as heme synthesis. This role of pyruvate carboxylase is termed *anaplerotic*, meaning to fill up.

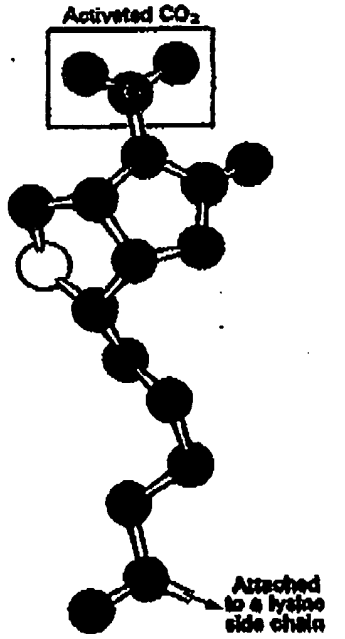
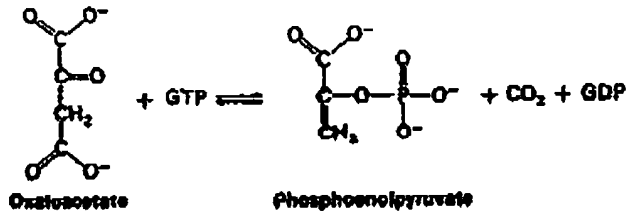


Figure 16-6
Molecular model of carboxybiotin.

OXALOACETATE IS SHUTTLED INTO THE CYTOSOL AND CONVERTED INTO PHOSPHOENOLPYRUVATE

Pyruvate carboxylase is a mitochondrial enzyme, whereas the other enzymes of gluconeogenesis are cytoplasmic. Oxaloacetate, the product of the pyruvate carboxylase reaction, is transported across the mitochondrial membrane in the form of *malate*. Oxaloacetate is reduced to malate inside the mitochondrion by an NADH-linked malate dehydrogenase. Malate is transported by a carrier across the mitochondrial membrane and is reoxidized to oxaloacetate by an NAD^+-linked malate dehydrogenase in the cytosol.

Oxaloacetate is simultaneously *decarboxylated* and *phosphorylated* by phosphoenolpyruvate carboxykinase in the cytosol.



The CO_2 that was added to pyruvate by pyruvate carboxylase comes off in this step. In fact, the phosphorylation reaction is made energetically feasible by the concomitant decarboxylation. *Decarboxylations often drive reactions that would otherwise be highly endergonic.* This device will be encountered again in fatty acid synthesis.

SIX HIGH-ENERGY PHOSPHATE BONDS ARE SPENT IN SYNTHESIZING GLUCOSE FROM PYRUVATE

The stoichiometry of gluconeogenesis is

$$2\ \text{Pyruvate} + 4\ \text{ATP} + 2\ \text{GTP} + 2\ \text{NADH} + 2\ H_2O \longrightarrow \text{glucose} + 4\ \text{ADP} + 2\ \text{GDP} + 6\ P_i + 2\ \text{NAD}^+ \qquad \Delta G^{\circ\prime} = -9\ \text{kcal/mol}$$

In contrast, the stoichiometry for the reversal of glycolysis is

$$2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ H_2O \longrightarrow \text{glucose} + 2\ \text{ADP} + 2\ P_i + 2\ \text{NAD}^+ \qquad \Delta G^{\circ\prime} = +20\ \text{kcal/mol}$$

Note that *six* high-energy phosphate bonds are used to synthesize glucose from pyruvate in gluconeogenesis, whereas only *two* molecules of ATP are generated in glycolysis in the conversion of glucose into pyruvate. Thus, the extra price of gluconeogenesis is four high-energy phosphate bonds per glucose synthesized from pyruvate. The four extra high-energy phosphate bonds are needed to turn an energetically unfavorable process (the reversal of glycolysis,

$\Delta G^{\circ\prime} = +20$ kcal/mol) into a favorable one (gluconeogenesis, $\Delta G^{\circ\prime} = -9$ kcal/mol). Another way of looking at this energetic difference between glycolysis and gluconeogenesis is to recall that the input of an ATP equivalent changes the equilibrium constant of a reaction by a factor of about 10^8 (p. 243). Hence, the input of four additional high-energy bonds in gluconeogenesis changes the equilibrium constant by a factor of 10^{32}, which makes the conversion of pyruvate into glucose thermodynamically feasible.

GLUCONEOGENESIS AND GLYCOLYSIS ARE RECIPROCALLY REGULATED

Gluconeogenesis and glycolysis are coordinated so that one pathway is relatively inactive while the other is highly active. If both sets of reactions were highly active at the same time, the net result would be the hydrolysis of four ~P (two ATP plus two GTP) per reaction cycle. Both glycolysis and gluconeogenesis are highly exergonic under cellular conditions, and so there is no thermodynamic barrier to such cycling. Rather, the activities of the distinctive enzymes of each pathway are controlled so that both pathways are not highly active at the same time. For example, AMP stimulates phosphofructokinase (p. 267), whereas it inhibits fructose 1,6-diphosphatase. Citrate has the reverse effect on these enzymes. *Consequently, phosphorylation of fructose 6-phosphate, the rate-limiting step in glycolysis, is enhanced when the energy charge of the cell is low. Conversely, fructose 1,6-diphosphate is hydrolyzed and gluconeogenesis is stimulated when the energy charge is high and citric acid cycle intermediates are abundant.* Pyruvate kinase (p. 265) and pyruvate carboxylase (p. 349) are also reciprocally regulated. Fructose 1,6-diphosphate stimulates and ATP inhibits pyruvate kinase, whereas acetyl CoA stimulates and ADP inhibits pyruvate carboxylase. Hence, *pyruvate flows to phosphoenolpyruvate and gluconeogenesis is favored when the liver cell is rich in fuel molecules and ATP.*

SUBSTRATE CYCLES AMPLIFY METABOLIC SIGNALS AND PRODUCE HEAT

A pair of reactions such as the phosphorylation of fructose 6-phosphate to fructose 1,6-diphosphate and its hydrolysis back to fructose 6-phosphate is called a *substrate cycle.* As already mentioned, both reactions are not simultaneously fully active in most cells because of reciprocal allosteric controls. However, isotope labeling studies have shown that phosphorylation of fructose 1,6-diphosphate occurs during gluconeogenesis. A limited degree of cycling also occurs in other pairs of opposed irreversible reactions. This cycling was regarded as an imperfection in metabolic control, and so substrate cycles have sometimes been called *futile cycles.* However, it now

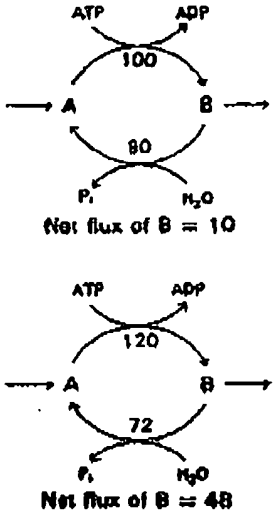


Figure 15-7
Example of an ATP-driven substrate cycle operating at two different rates. A small change in the rates of the two opposing reactions results in a large change in the *net* flux of product B.

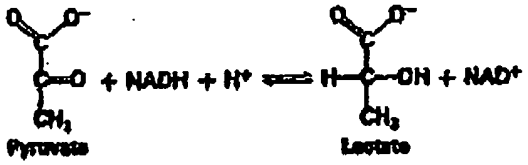
seems more likely that substrate cycles are biologically important. One possibility is that *substrate cycles amplify metabolic signals.* Suppose that the rate of conversion of A into B is 100 and of B into A is 90, giving an initial net flux of 10. Assume that an allosteric effector increases the A → B rate by 20% to 120 and reciprocally decreases the B → A rate by 20% to 72. The new net flux is 48, and so a 20% change in the rates of the opposing reactions has led to a 480% increase in the net flux. In the example shown in Figure 15-7, this amplification is achieved by the hydrolysis of ATP.

The other potential biological role of substrate cycles is the *generation of heat produced by the hydrolysis of ATP.* A striking example is provided by bumblebees, which have to maintain a thoracic temperature of about 30°C in order to fly. A bumblebee is able to maintain this high thoracic temperature and forage for food even when the ambient temperature is only 10°C because phosphofructokinase and fructose diphosphatase in its flight muscle are simultaneously highly active. This fructose diphosphatase is not inhibited by AMP, which suggests that the enzyme is specially designed for the generation of heat. In contrast, the honeybee has almost no fructose diphosphatase activity in its flight muscle and consequently cannot fly when the ambient temperature is low. Excessive heat production caused by too high a rate of cycling between fructose 6-phosphate and fructose 1,6-diphosphate can occur. Halothane, an anesthetic, produces this condition, called *malignant hyperthermia,* in a susceptible strain of pigs.

LACTATE FORMED BY CONTRACTING MUSCLE IS CONVERTED INTO GLUCOSE BY THE LIVER

A major raw material of gluconeogenesis is lactate produced by active skeletal muscle. The rate of production of pyruvate by glycolysis exceeds the rate of oxidation of pyruvate by the citric acid cycle in contracting skeletal muscle under anaerobic conditions. Moreover, the rate of formation of NADH in glycolysis in active muscle is greater than the rate of its oxidation by the respiratory chain. Continued glycolysis depends on the availability of NAD^+ for the oxidation of glyceraldehyde 3-phosphate. This is achieved by lactate dehydrogenase, which oxidizes NADH to NAD^+ as it reduces pyruvate to lactate.

Lactate is a dead end in metabolism. It must be converted back into pyruvate before it can be metabolized. The only purpose of the



reduction of pyruvate to lactate is to regenerate NAD^+ so that glycolysis can proceed in active skeletal muscle. *The formation of lactate buys time and shifts part of the metabolic burden from muscle to liver.*

The plasma membrane of most cells is highly permeable to lactate and pyruvate. Both substances diffuse out of active skeletal muscle into the blood and are carried to the liver. Much more lactate than pyruvate is carried because of the high $NADH/NAD^+$ ratio in contracting skeletal muscle. The lactate that enters the liver is oxidized to pyruvate, a reaction favored by the low $NADH/NAD^+$ in the cytosol of liver. Pyruvate is then converted into glucose by the gluconeogenic pathway in liver. Glucose then enters the blood and is taken up by skeletal muscle. Thus, *liver furnishes glucose to contracting skeletal muscle, which derives ATP from the glycolytic conversion of glucose into lactate. Glucose is then synthesized from lactate by the liver. These conversions constitute the Cori cycle* (Figure 15-8).

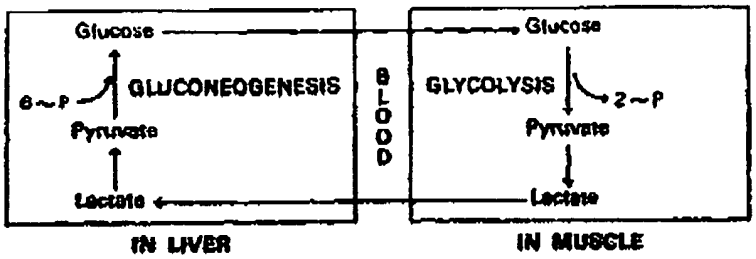


Figure 15-8
The Cori cycle. Lactate formed by active muscle is converted into glucose by the liver. This cycle shifts part of the metabolic burden of active muscle to the liver.

These conversions are facilitated by differences in the catalytic properties of lactate dehydrogenase enzymes in skeletal muscle and liver. Lactate dehydrogenase is a tetramer of 35-kdal subunits. There are two kinds of polypeptide chains called M and H, which can form five types of tetramers: M_4, M_3H, M_2H_2, M_1H_3, and H_4. These species are called *isoenzymes* (or isozymes). The M_4 isoenzyme has a much higher affinity for pyruvate than does the H_4 isoenzyme. The other isoenzymes have intermediate affinities. The principal isoenzyme in skeletal muscle and liver is M_4, whereas the main one in heart muscle is H_4. These isoenzymes have been studied intensively, but the reasons for the existence of multiple forms are an enigma.

SUMMARY

The pentose phosphate pathway generates NADPH and ribose 5-phosphate in the cytosol. NADPH is used in reductive biosyntheses, whereas ribose 5-phosphate is used in the synthesis of RNA, DNA, and nucleotide coenzymes. The pentose phosphate pathway starts with the dehydrogenation of glucose 6-phosphate to form a lactone, which is hydrolyzed to give 6-phosphogluconate and then oxidatively decarboxylated to yield ribulose 5-phosphate. $NADP^+$ is the electron acceptor in both of these oxidations. The last step is the isomerization of ribulose 5-phosphate (a ketose) to ribose 5-phosphate (an aldose). A different mode of the pathway is active when cells need much more NADPH than ribose 5-phosphate. Under these conditions, ribose 5-phosphate is converted into glyceraldehyde 3-phosphate and fructose 6-phosphate by transketolase and transaldolase. Transketolase contains TPP as its prosthetic group. These enzymes create a reversible link between the pentose phosphate pathway and glycolysis. Xylulose 5-phosphate, sedoheptulose 7-phosphate, and erythrose 4-phosphate are intermediates in these interconversions. In this way, twelve NADPH can be generated for each glucose 6-phosphate that is completely oxidized to CO_2. Only the nonoxidative branch of the pathway is active when much more ribose 5-phosphate than NADPH needs to be synthesized. Under these conditions, fructose 6-phosphate and glyceraldehyde 3-phosphate (formed by the glycolytic pathway) are converted into ribose 5-phosphate without the formation of NADPH. Alternatively, ribose 5-phosphate formed by the oxidative branch can be converted into pyruvate through fructose 6-phosphate and glyceraldehyde 3-phosphate. In this mode, ATP and NADPH are generated, and five of the six carbons of glucose 6-phosphate emerge in pyruvate. The interplay of the glycolytic and pentose phosphate pathways enables the levels of NADPH, of ATP, and of building blocks such as ribose 5-phosphate and pyruvate to be continuously adjusted to meet cellular needs.

Gluconeogenesis is the synthesis of glucose from noncarbohydrate sources, such as lactate, amino acids, and glycerol. Several of the reactions that convert pyruvate, a major entry point, into glucose are common to glycolysis. However, gluconeogenesis requires four new reactions to bypass the essential irreversibility of the corresponding reactions in glycolysis. Pyruvate is carboxylated to oxaloacetate in mitochondria, which in turn is decarboxylated and phosphorylated to phosphoenolpyruvate in the cytosol. Two high-energy phosphate bonds are consumed in these reactions, which are catalyzed by pyruvate carboxylase and phosphoenolpyruvate carboxykinase. Pyruvate carboxylase contains a biotin prosthetic group. The other distinctive reactions of gluconeogenesis are the hydrolyses of fructose 1,6-diphosphate and glucose 6-phosphate, which are catalyzed by specific phosphatases. Gluconeogenesis and glycolysis are reciprocally regulated so that one pathway is relatively inactive while the other is highly active.

CHAPTER 21

BIOSYNTHESIS OF AMINO ACIDS AND HEME

This chapter deals with the biosynthesis of amino acids and some molecules derived from them. The flow of nitrogen into amino acids will be considered first. This process starts with the reduction of N_2 to NH_4^+ by nitrogen-fixing microorganisms. NH_4^+ is then assimilated into amino acids by way of glutamate and glutamine, the two pivotal molecules in nitrogen metabolism. Of the basic set of twenty amino acids, ten are synthesized from citric acid cycle and other major metabolic intermediates by quite simple reactions. We will consider these biosyntheses and examine the biosyntheses of the aromatic amino acids and of histidine as examples of amino acids formed by more complex routes. In fact, humans must obtain the latter group of ten amino acids from their diets, and so they are called essential amino acids. Two interesting carriers participate in these reactions: tetrahydrofolate, a highly versatile carrier of activated one-carbon units at three oxidation stages, and *S*-adenosylmethionine, the major methyl donor. The regulation of amino acid metabolism is another important area of inquiry. We will take a look at glutamine synthetase, which exemplifies some general principles. The final section of this chapter is concerned with the synthesis and degradation of heme.

Figure 21-2
The nodules in the root system of the soybean are the sites of nitrogen fixation by *Rhizobium* bacteria. [Courtesy of Dr. Joe C. Burton, Nitragin Company, Inc.]

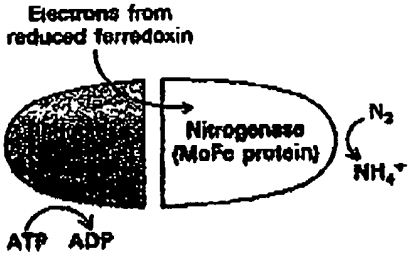


Figure 21-3
Schematic diagram of the nitrogenase complex. The reductase dissociates from the nitrogenase component before N_2 is converted into NH_4^+.

MICROORGANISMS USE ATP AND A POWERFUL REDUCTANT TO CONVERT N_2 INTO NH_4^+

The nitrogen atoms of amino acids, purines, pyrimidines, and other biomolecules come from NH_4^+. Higher organisms are unable to convert N_2 into organic form. Rather, this conversion—called *nitrogen fixation*—is carried out by bacteria and blue-green algae. Some of these microorganisms—namely, the *Rhizobium* bacteria—invade the roots of leguminous plants and form root nodules, in which nitrogen fixation takes place (Figure 21-2). The relation between the bacteria and plant is symbiotic. The amount of N_2 fixed by microorganisms has been estimated to be about 2×10^{11} kg per year.

The N≡N bond, which has a bond energy of 225 kcal/mol, is highly resistant to chemical attack. Indeed, Lavoisier named it "azote," meaning "without life," because it is quite unreactive. The industrial process for nitrogen fixation, devised by Fritz Haber in 1910 and currently used in fertilizer factories,

$$N_2 + 3\,H_2 \rightleftharpoons 2\,NH_3$$

is typically carried out over an iron catalyst at about 500°C and a pressure of 300 atm. It is not surprising, then, that the biological process of nitrogen fixation requires a complex enzyme. The *nitrogenase complex*, which carries out this process, consists of two kinds of protein components: a *reductase*, which provides electrons with high reducing power, and a *nitrogenase*, which uses these electrons to reduce N_2 to NH_4^+ (Figure 21-3). Each component is an *iron-sulfur protein*, in which iron is bonded to the sulfur atom of a cysteine residue and to inorganic sulfide (p. 312). The nitrogenase component of the complex also contains one or two *molybdenums* and so it has been known as the *MoFe protein*. It has the subunit structure $\alpha_2\beta_2$ and a mass of about 200 kdal. The reductase (also called the *Fe protein*) consists of two identical polypeptides and has a mass of about 65 kdal. In the nitrogenase complex, one or two Fe proteins are associated with a MoFe protein.

The conversion of N_2 into NH_4^+ by the nitrogenase complex *requires ATP and a powerful reductant*. In most nitrogen-fixing microorganisms, the source of high-potential electrons in this *six-electron reduction* is *reduced ferredoxin*, an electron carrier previously encountered in photosynthesis (p. 439). Whether reduced ferredoxin is then regenerated by photosynthetic or oxidative processes depends on the particular species. The stoichiometry of the reaction catalyzed by the nitrogenase complex is

$$N_2 + 6\,e^- + 12\,ATP + 12\,H_2O \longrightarrow 2\,NH_4^+ + 12\,ADP + 12\,P_i + 4\,H^+$$

Recent studies of nitrogenase suggest the following reaction sequence. First, reduced ferredoxin transfers its electrons to the re-

ductase component of the complex. Second, ATP then binds to the reductase and shifts its redox potential from −0.29 to −0.40 V by altering its conformation. This enhancement of the reducing power of the reductase enables it to transfer its electrons to the nitrogenase component. Third, electrons are transferred, ATP is hydrolyzed, and the reductase dissociates from the nitrogenase component. Finally, N_2 bound to the nitrogenase component of the complex is reduced to NH_4^+.

Sources of energy for the chemical production of ammonia by the Haber process are becoming scarcer and more costly, and so there is much current interest in enhancing nitrogen fixation by microorganisms. One potential approach is to insert the genes for nitrogen fixation into nonleguminous plants, such as cereals. A difficulty that must be overcome is that the nitrogenase complex is exquisitely sensitive to inactivation by O_2. Leguminous plants maintain a very low concentration of free O_2 in their root nodules by binding O_2 to *leghemoglobin*. Another challenge that must be met in the formation of new nitrogen-fixing species is the requirement for a very high rate of ATP formation. In fact, nitrogen-fixing bacteria in the roots of pea plants consume nearly a fifth of all the ATP generated by the plant. A complementary approach is to increase the rate of nitrogen fixation by blue-green algae, which generate their own ATP by photosynthesis and thus are not dependent on an energy-yielding symbiotic relation.

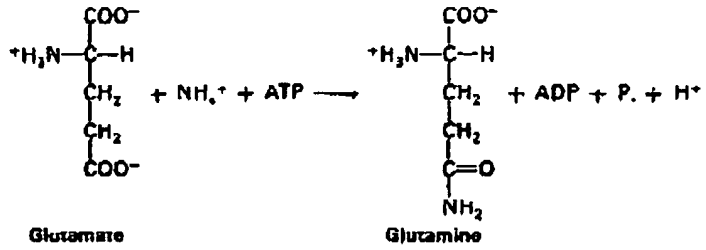
NH_4^+ IS ASSIMILATED INTO AMINO ACIDS BY WAY OF GLUTAMATE AND GLUTAMINE

The next step in the assimilation of nitrogen into biomolecules is the entry of NH_4^+ into amino acids. *Glutamate* and *glutamine* play pivotal roles in this regard. The α-amino group of most amino acids comes from the α-amino group of glutamate by transamination. Glutamine, the other major nitrogen donor, contributes its side-chain nitrogen in the biosynthesis of a wide range of important compounds.

Glutamate is synthesized from NH_4^+ and α-ketoglutarate, a citric acid cycle intermediate, by the action of *glutamate dehydrogenase*. This enzyme has already been encountered in the degradation of amino acids (p. 408). In the biosynthetic direction, NADPH is the reductant, whereas NAD^+ is the oxidant in the catabolic direction.

$$NH_4^+ + \text{α-ketoglutarate} + NADPH + H^+ \rightleftharpoons \text{L-glutamate} + NADP^+ + H_2O$$

Ammonium ion is incorporated into glutamine by the action of *glutamine synthetase*. This amidation is driven by the hydrolysis of ATP.



The regulation of glutamine synthetase plays a critical role in controlling nitrogen metabolism, as will be discussed shortly.

Glutamate dehydrogenase and glutamine synthetase are present in all organisms. Most procaryotes also contain *glutamate synthase*, which catalyzes the reductive amination of α-ketoglutarate. The nitrogen donor in this reaction is glutamine, and so two molecules of glutamate are formed.

$$\alpha\text{-Ketoglutarate} + \text{glutamine} + \text{NADPH} + \text{H}^+ \longrightarrow 2\ \text{glutamate} + \text{NADP}^+$$

When NH_4^+ is limiting, most of the glutamate is made by the sequential action of glutamine synthetase and glutamate synthase. The sum of these reactions is

$$NH_4^+ + \alpha\text{-ketoglutarate} + \text{NADPH} + \text{ATP} \longrightarrow \text{L-glutamate} + \text{NADP}^+ + \text{ADP} + P_i$$

Note that this stoichiometry differs from that of the glutamate dehydrogenase reaction in that an ATP is hydrolyzed. Why is this more expensive pathway sometimes used by *E. coli*? The answer is that the K_M of glutamate dehydrogenase for NH_4^+ is high (~1 mM), and so this enzyme is not saturated when NH_4^+ is limiting. In contrast, glutamine synthetase has very high affinity for NH_4^+.

Table 21-1
Basic set of twenty amino acids

Nonessential	*Essential*
Alanine	Arginine
Asparagine	Histidine
Aspartate	Isoleucine
Cysteine	Leucine
Glutamate	Lysine
Glutamine	Methionine
Glycine	Phenylalanine
Proline	Threonine
Serine	Tryptophan
Tyrosine	Valine

AMINO ACIDS ARE SYNTHESIZED FROM CITRIC ACID CYCLE AND OTHER MAJOR METABOLIC INTERMEDIATES

Thus far, we have considered the conversion of N_2 into NH_4^+ and the assimilation of NH_4^+ into glutamate and glutamine. We turn now to the biosynthesis of the other amino acids. Bacteria such as *E. coli* can synthesize the entire basic set of twenty amino acids, whereas humans can make only half of them. The amino acids that must be supplied in the diet are called *essential*, whereas the others are termed *nonessential* (Table 21-1). *These designations refer to the needs of an organism under a particular set of conditions.* For example, enough arginine is synthesized by the urea cycle to meet the needs of an adult but not those of a growing child. A deficiency of even one amino acid results in a *negative nitrogen balance*. In this state, more

protein is degraded than is synthesized, and so more nitrogen is excreted than is ingested.

The pathways for the biosynthesis of amino acids are diverse. However, they have an important common feature: *their carbon skeletons come from glycolytic, pentose phosphate pathway, or citric acid cycle intermediates.* A further simplification is that there are only *six biosynthetic families* (Figure 21-4).

The nonessential amino acids are synthesized by quite simple reactions, whereas the pathways for the formation of the essential amino acids are quite complex. For example, the nonessential amino acids *alanine* and *aspartate* are synthesized in a single step from pyruvate and oxaloacetate, respectively. Each acquires its amino group from glutamate in a transamination reaction in which pyridoxal phosphate is the cofactor (p. 410):

$$\text{Pyruvate} + \text{glutamate} \rightleftharpoons \text{alanine} + \alpha\text{-ketoglutarate}$$

$$\text{Oxaloacetate} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha\text{-ketoglutarate}$$

Asparagine is then synthesized by the amidation of aspartate:

$$\text{Aspartate} + NH_4^+ + \text{ATP} \longrightarrow \text{asparagine} + \text{AMP} + PP_i + H^+$$

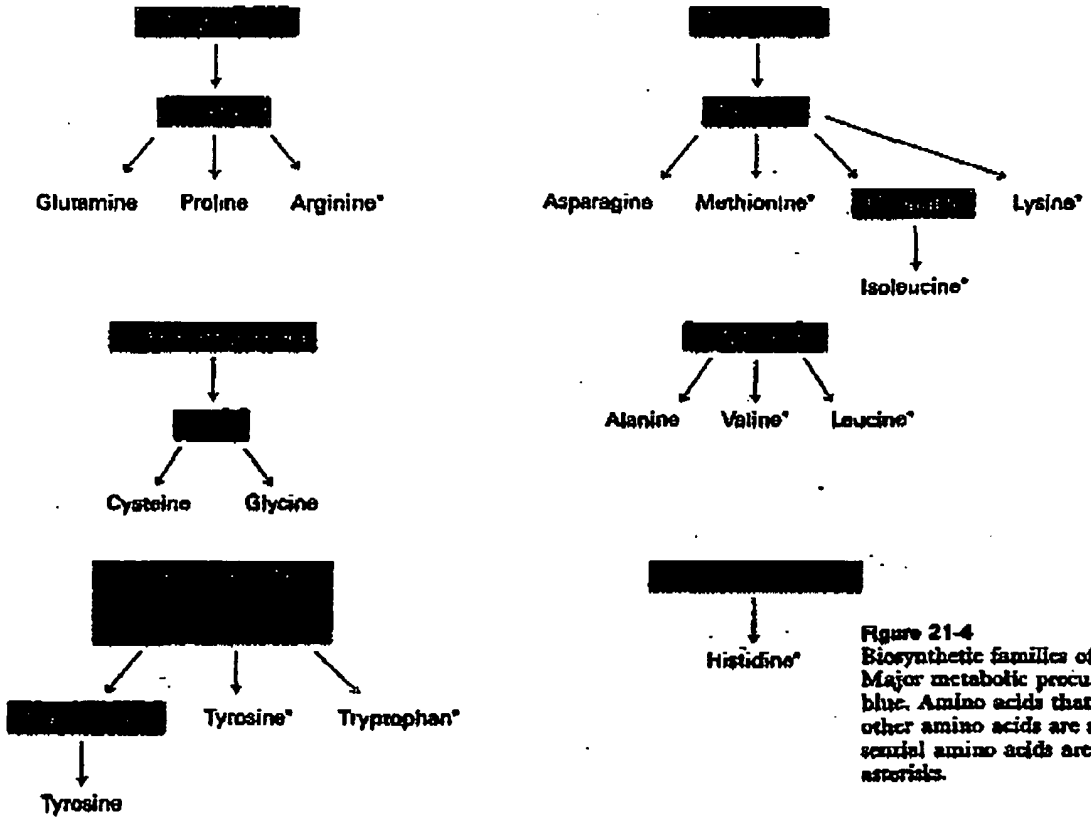


Figure 21-4
Biosynthetic families of amino acids. Major metabolic precursors are shaded blue. Amino acids that give rise to other amino acids are shaded red. Essential amino acids are designated by asterisks.

In mammals, the nitrogen donor in the synthesis of asparagine is glutamine rather than NH_4^+.

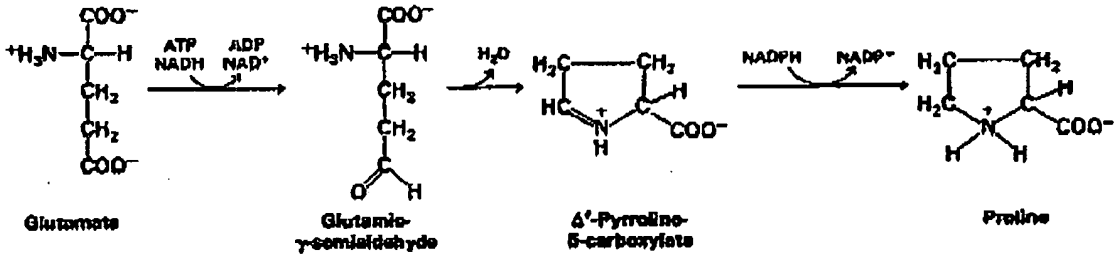
Another one-step synthesis of a nonessential amino acid is the hydroxylation of phenylalanine (an essential amino acid) to *tyrosine*, a reaction occurring in mammals.

$$\text{Phenylalanine} + O_2 + \text{NADPH} + H^+ \longrightarrow \text{tyrosine} + \text{NADP}^+ + H_2O$$

This reaction is catalyzed by phenylalanine hydroxylase, a monooxygenase discussed previously (p. 424). It is noteworthy that tyrosine is an essential amino acid in individuals lacking this enzyme.

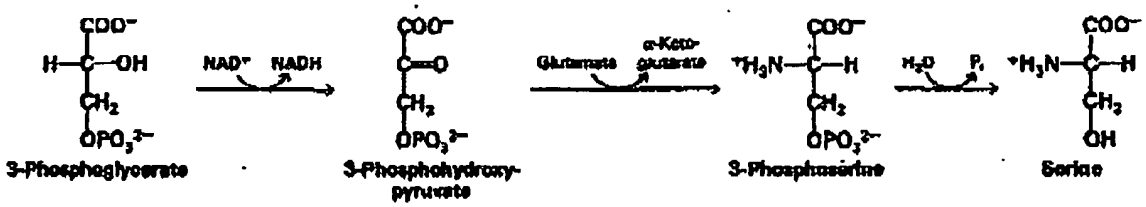
GLUTAMATE IS THE PRECURSOR OF GLUTAMINE AND PROLINE

The synthesis of glutamate by the reductive amination of α-ketoglutarate has already been discussed (p. 487), as has the conversion of glutamate into glutamine (p. 488). Glutamate is the precursor of one other nonessential amino acid, proline. First, the γ-carboxyl group of glutamate reacts with ATP to form an acyl phosphate. This mixed anhydride is then reduced by NADPH to an aldehyde. Glutamic γ-semialdehyde cyclizes with a loss of H_2O to give Δ¹-pyrroline-5-carboxylate, which is reduced by NADPH to yield proline.



SERINE IS SYNTHESIZED FROM 3-PHOSPHOGLYCERATE

Serine is synthesized from 3-phosphoglycerate, an intermediate in glycolysis. The first step is an oxidation to 3-phosphohydroxypyruvate. This α-keto acid is transaminated to 3-phosphoserine, which is then hydrolyzed to yield serine.



Alternatively, hydrolysis of the phosphate group may precede oxidation and transamination:

3-Phosphoglycerate ⟶ glycerate ⟶ hydroxypyruvate ⟶ serine

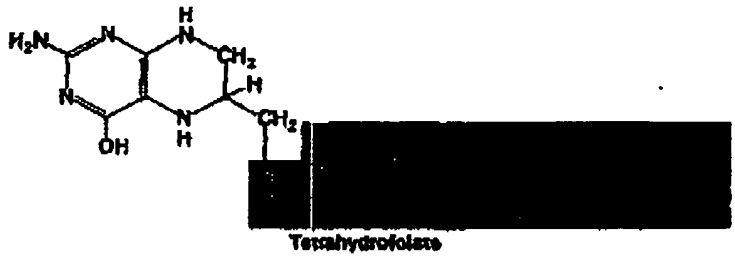
Serine is the precursor of *glycine* and *cysteine*. In the formation of glycine, the side-chain β-carbon atom of serine is transferred to *tetrahydrofolate*, a carrier of one-carbon units that will be discussed shortly.

Serine + tetrahydrofolate ⇌ glycine + methylenetetrahydrofolate + H_2O

This conversion is catalyzed by *serine transhydroxymethylase*, a pyridoxal phosphate (PLP) enzyme. The bond between the α- and β-carbon atoms of serine is labilized by the formation of a Schiff base between serine and PLP. The β-carbon atom of serine is then transferred to tetrahydrofolate. Glycine can also be formed from CO_2, NH_4^+, and methylenetetrahydrofolate in a reaction catalyzed by *glycine synthase*. The conversion of serine into cysteine requires the substitution of a sulfur atom derived from methionine for the side-chain oxygen atom. This reaction sequence will be presented after one-carbon metabolism has been considered.

TETRAHYDROFOLATE CARRIES ACTIVATED ONE-CARBON UNITS AT SEVERAL OXIDATION LEVELS

Tetrahydrofolate (also called tetrahydropteroylglutamate), a highly versatile carrier of activated one-carbon units, consists of three groups: a substituted pteridine, *p*-aminobenzoate, and glutamate. Mammals are unable to synthesize a pteridine ring. They obtain tetrahydrofolate from their diets or from microorganisms in their intestinal tracts.

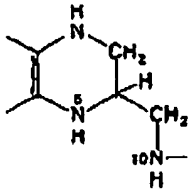


Tetrahydrofolate

The one-carbon group carried by tetrahydrofolate is bonded to its N-5 or N-10 nitrogen atom (denoted as N^5 and N^{10}) or to both. This unit can exist in three oxidation states (Table 21-2). The most reduced form carries a *methyl* group, whereas the intermediate form carries a *methylene* group. The most oxidized forms carry a *methenyl*, *formyl*, or *formimino* group. The most oxidized one-carbon unit, CO_2,

Table 21-2
One-carbon groups carried by tetrahydrofolate

Oxidation state	*Group*	
Most reduced	$-CH_3$	Methyl
Intermediate	$-CH_2-$	Methylene
Most oxidized	—CHO	Formyl
	—CHNH	Formimino
	—CH=	Methenyl



Reactive part of tetrahydrofolate

is carried by biotin (p. 348) rather than by tetrahydrofolate.

These one-carbon units are interconvertible (Figure 21-5). N^5, *N^{10}-Methylene*tetrahydrofolate can be reduced to *N^5-methyl*tetrahydrofolate or oxidized to *N^5-methenyl*tetrahydrofolate. N^5, *N^{10}-Methenyl*tetrahydrofolate can be converted into *N^5-formimino*tetrahydrofolate and *N^{10}-formyl*tetrahydrofolate, which are at the same oxidation level. N^{10}-Formyltetrahydrofolate can also be synthesized from formate and ATP:

$$\text{Formate} + \text{ATP} + \text{tetrahydrofolate} \rightleftharpoons N^{10}\text{-formyltetrahydrofolate} + \text{ADP} + P_i$$

These tetrahydrofolate derivatives serve as donors of one-carbon units in a variety of biosyntheses. Methionine is synthesized from homocysteine by transfer of the methyl group of N^5-methyltetrahydrofolate, as will be discussed shortly. Some of the carbon atoms of *purines* are derived

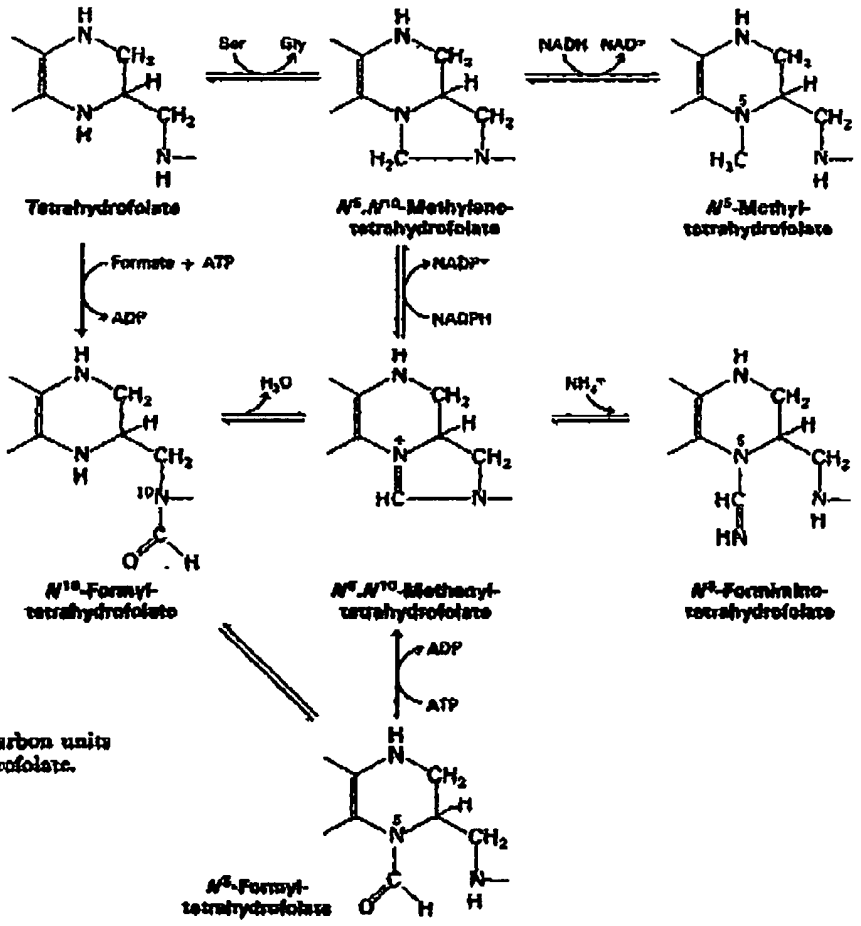


Figure 21-5
Conversions of one-carbon units attached to tetrahydrofolate.

from the N^5, N^{10}-methenyl and the N^{10}-formyl derivatives of tetrahydrofolate. The methyl group of *thymine*, a pyrimidine, comes from N^5, N^{10}-methylenetetrahydrofolate. This tetrahydrofolate derivative also donates a one-carbon unit in the synthesis of *glycine* from CO_2 and NH_4^+, a reaction catalyzed by glycine synthase.

$$CO_2 + NH_4^+ + N^5, N^{10}\text{-methylenetetrahydrofolate} + NADH \rightleftharpoons \text{glycine} + \text{tetrahydrofolate} + NAD^+$$

Thus, one-carbon units at each of the three oxidation levels are utilized in biosyntheses. In turn, *tetrahydrofolate serves as an acceptor of one-carbon units in degradative reactions*. The major source of one-carbon units is the conversion of serine into glycine, which yields N^5, N^{10}-methylenetetrahydrofolate, as previously mentioned. Serine can be derived from 3-phosphoglycerate (p. 490), and so *this pathway enables one-carbon units to be formed de novo from carbohydrate*. The breakdown of *histidine* yields *N*-formiminoglutamate, which transfers its formimino group to tetrahydrofolate to form the N^5-derivative.

S-ADENOSYLMETHIONINE IS THE MAJOR DONOR OF METHYL GROUPS

Tetrahydrofolate can carry a methyl group on its N^5-atom, but its transfer potential is not sufficiently high. Rather, the activated methyl donor in most biosyntheses is *S-adenosylmethionine*, which has already been encountered in the conversion of phosphatidyl ethanolamine into phosphatidyl choline (p. 459). *S*-Adenosylmethionine is synthesized by the transfer of an adenosyl group from ATP to the sulfur atom of methionine. The methyl group of the methionine unit is activated by the positive charge on the adjacent sulfur atom, which makes it much more reactive than N^5-methyltetrahydrofolate.

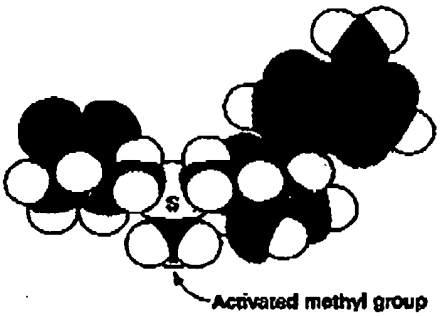
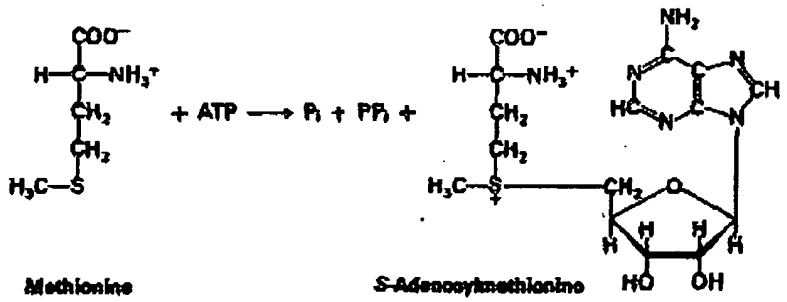
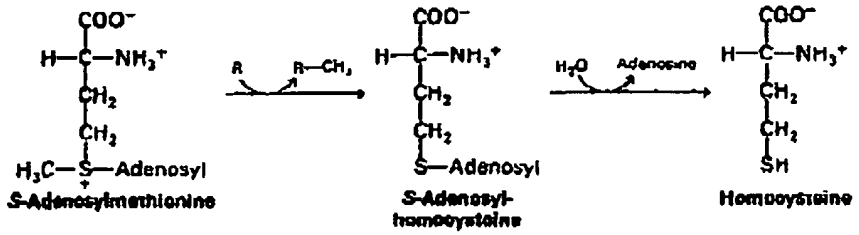


Figure 21-6
Space-filling model of *S*-adenosylmethionine.

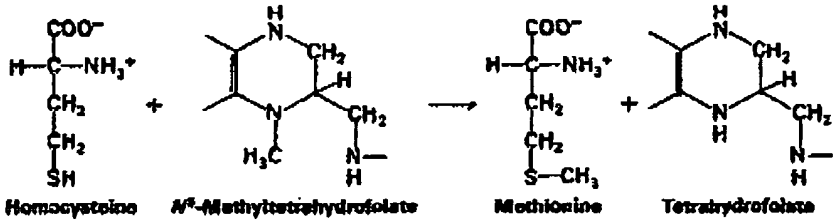


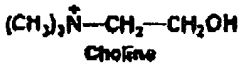
The synthesis of *S*-adenosylmethionine is unusual in that the triphosphate group of ATP is split into pyrophosphate and orthophosphate. Pyrophosphate is then hydrolyzed. Thus, all of the phosphorus–oxygen bonds in ATP are split in this activation reaction, which markedly enhances the reactivity of the methyl group.

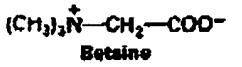
S-Adenosylhomocysteine is formed when the methyl group of *S*-adenosylmethionine is transferred to an acceptor such as phosphatidyl ethanolamine. *S*-Adenosylhomocysteine is then hydrolyzed to *homocysteine* and adenosine.



Methionine can be regenerated by the transfer of a methyl group from N^5-methyltetrahydrofolate, a reaction catalyzed by *homocysteine methyltransferase.*







This transfer of a methyl group is mediated by *methylcobalamin,* the coenzyme of homocysteine transmethylase. In fact, this reaction and the rearrangement of L-methylmalonyl CoA to succinyl CoA (p. 419) are the only two known Vitamin B_{12}-dependent reactions in mammals. Alternatively, homocysteine can be methylated to methionine by donors such as *betaine,* an oxidation product of choline.

These reactions constitute the *activated methyl cycle* (Figure 21-7). Methyl groups enter the cycle in the conversion of homocysteine into methionine and are then made highly reactive by the expenditure of 3 ~P. The high transfer potential of the methyl group in *S*-adenosylmethionine enables it to be transferred to a wide variety of acceptors, such as the amino group of the neurotransmitter norepinephrine (p. 895) and a glutamate residue of a regulatory protein in chemotaxis (p. 909).

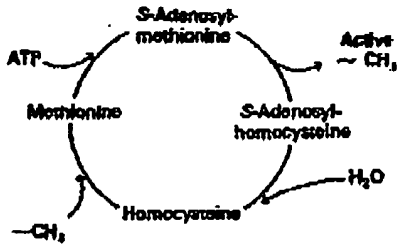


Figure 21-7
Activated methyl cycle.

CYSTEINE IS SYNTHESIZED FROM SERINE AND HOMOCYSTEINE

Homocysteine is an intermediate in the synthesis of cysteine, in addition to being a precursor of methionine in the activated methyl

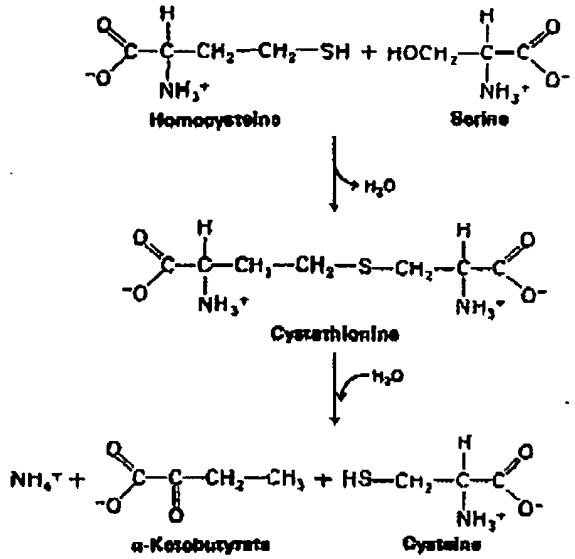


Figure 21-8
Synthesis of cysteine.

cycle. Serine and homocysteine condense to form *cystathionine* (Figure 21-8). This reaction is catalyzed by cystathionine synthetase, a PLP enzyme. Cystathionine is then deaminated and cleaved to cysteine and α-ketobutyrate by *cystathioninase*, another PLP enzyme. The net reaction is:

$$\text{Homocysteine} + \text{serine} \longrightarrow \text{cysteine} + \alpha\text{-ketobutyrate}$$

Note that the sulfur atom of cysteine is derived from homocysteine, whereas the carbon skeleton comes from serine.

This completes our consideration of the biosynthesis of the nonessential amino acids. The formation of tyrosine by the hydroxylation of phenylalanine was discussed earlier (p. 424).

SHIKIMATE AND CHORISMATE ARE INTERMEDIATES IN THE BIOSYNTHESIS OF AROMATIC AMINO ACIDS

We turn now to the biosynthesis of essential amino acids, which are formed by much more complex routes than are the nonessential amino acids. Two pathways have been selected for discussion here—those of the aromatic amino acids and of histidine.

Phenylalanine, tyrosine, and tryptophan are synthesized by a common pathway in *E. coli* (Figure 21-9). The initial step is the condensation of phosphoenolpyruvate (a glycolytic intermediate) and erythrose 4-phosphate (a pentose phosphate pathway intermediate). The resulting C_7 open-chain sugar loses its phosphoryl group and cyclizes to 5-dehydroquinate. Dehydration then yields 5-dehydroshikimate, which is reduced by NADPH to *shikimate* (Figure 21-10). A second molecule of phosphoenolpyruvate then condenses with 5-phosphoshikimate to give an intermediate that loses its phosphoryl group, which yields *chorismate*.

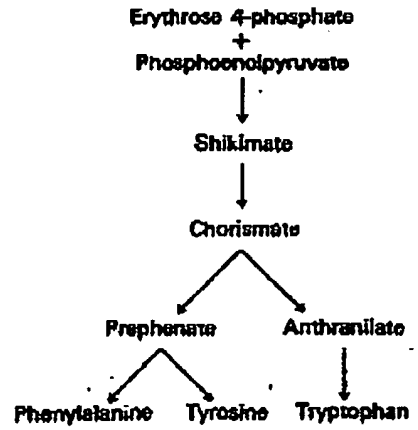


Figure 21-9
Pathway for the biosynthesis of aromatic amino acids in *E. coli*.

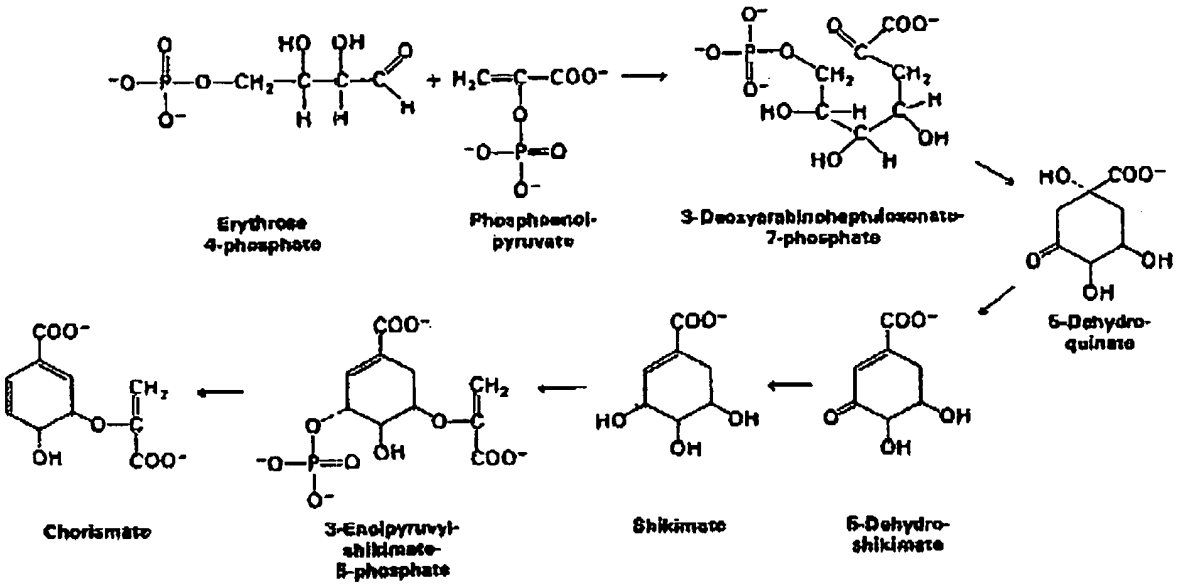
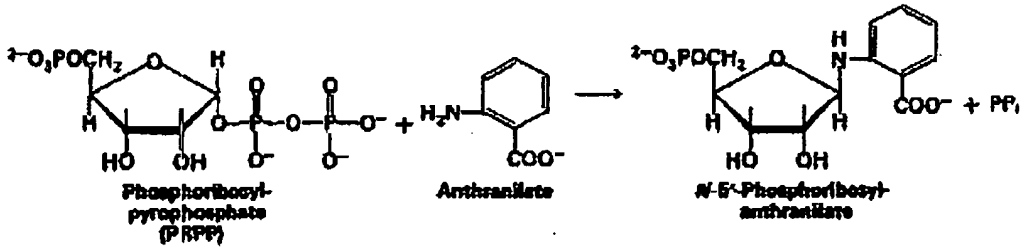


Figure 21-10
Synthesis of chorismate, an intermediate in the biosynthesis of phenylalanine, tyrosine, and tryptophan in *E. coli*.

The pathway bifurcates at chorismate. Let us first follow the *prephenate branch* (Figure 21-11). A mutase converts chorismate into prephenate, the immediate precursor of the aromatic ring of phenylalanine and tyrosine. Dehydration and decarboxylation yield *phenylpyruvate*. Alternatively, prephenate can be oxidatively decarboxylated to yield *p-hydroxyphenylpyruvate*. These α-keto acids are then transaminated to yield *phenylalanine* and *tyrosine*, respectively.

The branch starting with *anthranilate* leads to the synthesis of *tryptophan*. Chorismate acquires an amino group from the side chain of glutamine to form anthranilate. In fact, *glutamine serves as an amino donor in many biosynthetic reactions*. Anthranilate then condenses with *phosphoribosylpyrophosphate* (PRPP), *an activated form of ribose phosphate*. PRPP is also a key intermediate in the synthesis of histidine, purine nucleotides, and pyrimidine nucleotides (p. 514). The C-1 atom of ribose 5-phosphate becomes bonded to the nitrogen atom of anthranilate in a reaction that is driven by the hydrolysis of pyrophosphate.



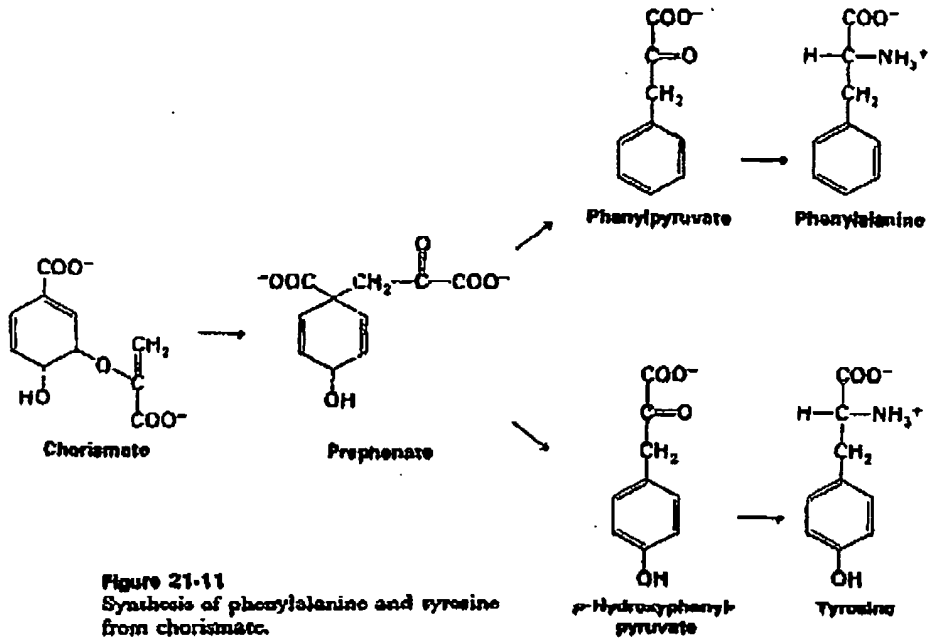


Figure 21-11
Synthesis of phenylalanine and tyrosine from chorismate.

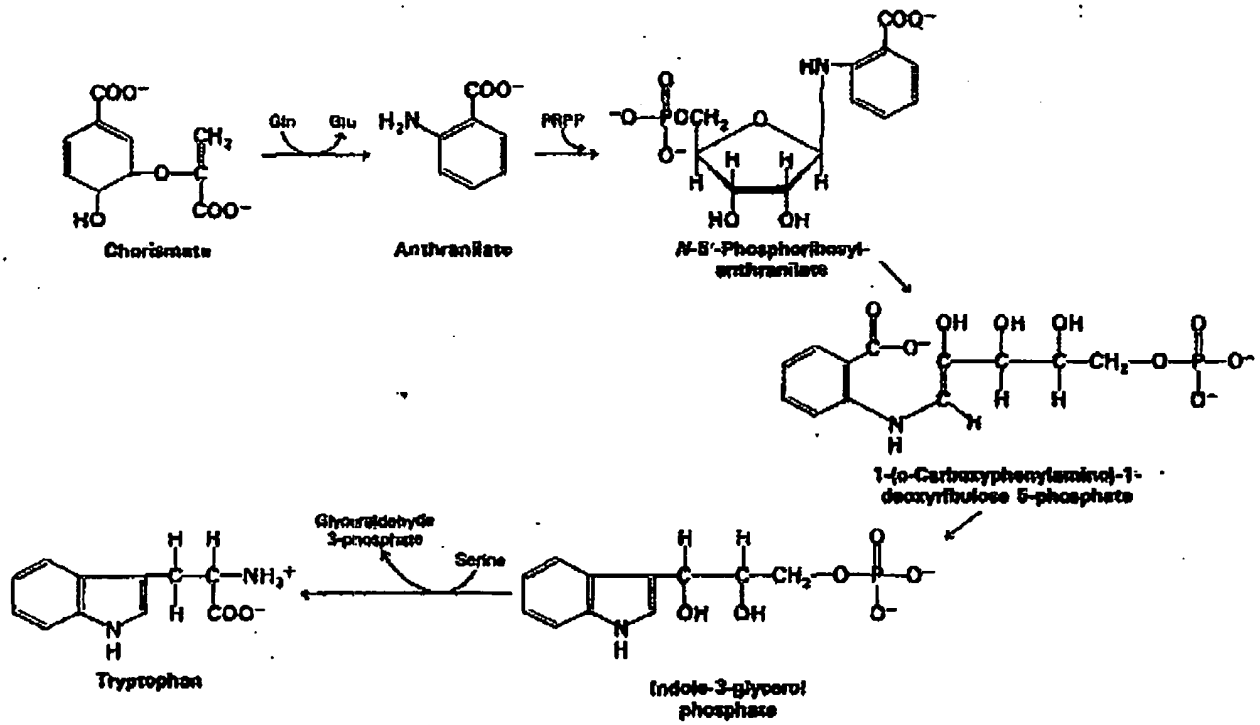


Figure 21-12
Synthesis of tryptophan from chorismate.

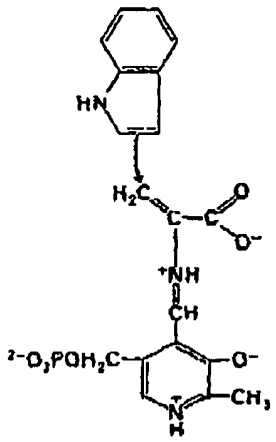


Figure 21-13
Proposed intermediate in the synthesis of tryptophan. Serine forms a Schiff base with PLP on a β chain and is then dehydrated to give the Schiff base of aminoacrylate (shown in red). This enzyme-bound intermediate is attacked by indole, the product of the partial reaction catalyzed by the α subunit, to give tryptophan.

The ribose moiety of phosphoribosylanthranilate undergoes rearrangement (Figure 21-12) to yield 1-(o-carboxyphenylamino)-1-deoxyribulose 5-phosphate. This intermediate is dehydrated and decarboxylated to form *indole-3-glycerol phosphate*. Finally, indole-3-glycerol phosphate reacts with serine to form *tryptophan*. The glycerol phosphate side chain of indole-3-glycerol phosphate is replaced by the carbon skeleton and amino group of serine. This reaction is catalyzed by tryptophan synthetase.

Tryptophan synthetase of *E. coli* has the subunit structure $\alpha_2\beta_2$. The enzyme can be dissociated into two α subunits and a β_2 subunit. The isolated subunits catalyze partial reactions that lead to the synthesis of tryptophan:

$$\text{Indole-3-glycerol phosphate} \xrightarrow{\alpha \text{ subunit}} \text{indole} + \text{glyceraldehyde 3-phosphate}$$

$$\text{Indole} + \text{serine} \xrightarrow{\beta_2 \text{ subunit}} \text{tryptophan} + H_2O$$

Each active site on the β_2 subunit contains a PLP prosthetic group. *The catalytic properties of the α and β_2 subunits are markedly altered on the formation of the $\alpha_2\beta_2$ complex.* The rates of the partial reactions are more than ten times as great for the $\alpha_2\beta_2$ complex as for the isolated subunits. Furthermore, the $\alpha_2\beta_2$ complex synthesizes tryptophan by a concerted mechanism. Indole formed by the first partial reaction reacts immediately with serine, so that indole is not released from the $\alpha_2\beta_2$ complex. Thus, the catalytic properties of a multisubunit enzyme can be altered by interactions between its subunits.

HISTIDINE IS SYNTHESIZED FROM ATP, PRPP, AND GLUTAMINE

The pathway for histidine biosynthesis in *E. coli* and *Salmonella* contains many complex and novel features (Figure 21-14). The reaction sequence starts with the condensation of ATP and PRPP, in which N-1 of the purine ring becomes bonded to C-1 of the ribose unit of PRPP. In fact, five carbon atoms of histidine come from PRPP. The adenine unit of ATP provides a nitrogen and a carbon atom of the imidazole ring of histidine. The other nitrogen atom of the imidazole ring comes from the side chain of glutamine. A noteworthy aspect of this pathway is that 5-aminoimidazole-4-carboxamide ribonucleotide, which is produced in the cleavage reaction that forms the imidazole ring, is an intermediate in purine biosynthesis (p. 515). Thus, histidine biosynthesis and purine biosynthesis are linked.

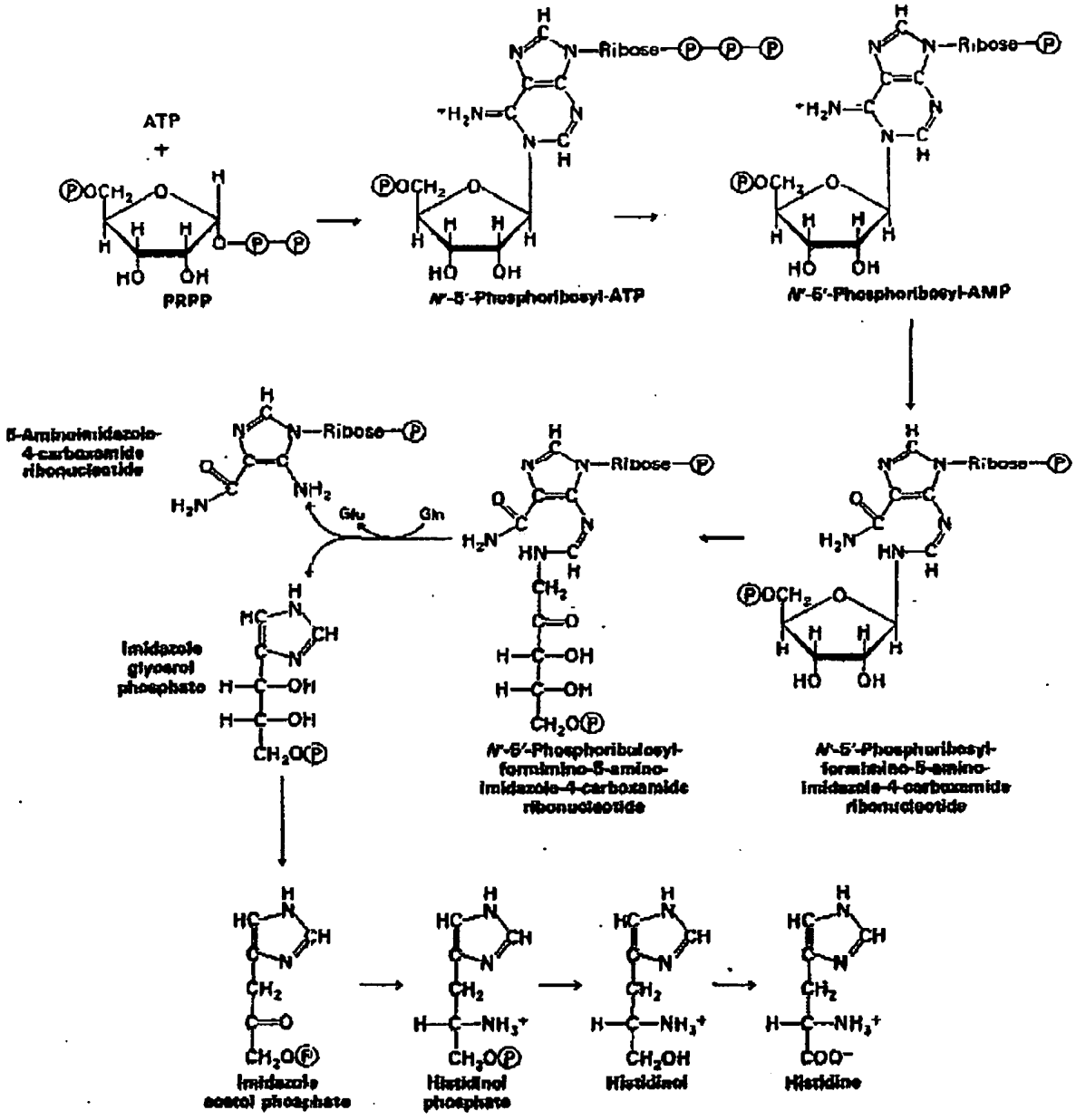
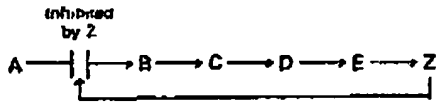


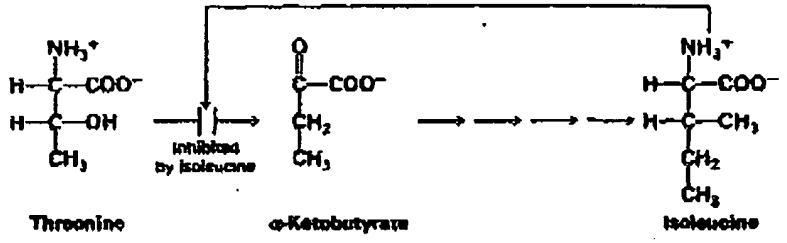
Figure 21-14
Pathway for the biosynthesis of histidine in *E. coli* and *Salmonella* (Ⓟ denotes a phosphoryl group).



AMINO ACID BIOSYNTHESIS IS REGULATED BY FEEDBACK INHIBITION

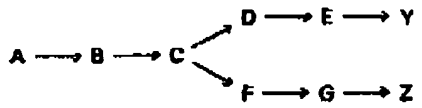
The rate of synthesis of amino acids depends mainly on the *amounts* of the biosynthetic enzymes and on their enzymatic *activities*. We will now consider the control of enzymatic activity. The regulation of enzyme synthesis will be discussed in Chapter 28.

The first irreversible reaction in a biosynthetic pathway, called the committed step, is usually an important regulatory site. *The final product of the pathway (Z) often inhibits the enzyme that catalyzes the committed step (A → B).* This kind of control is essential for the conservation of building blocks and metabolic energy. The first example of this important principle of metabolic control came from studies of the biosynthesis of isoleucine in *E. coli*. The dehydration and deamination of threonine to α-ketobutyrate is the committed step in the synthesis of isoleucine. *Threonine deaminase,* the PLP enzyme that catalyzes this reaction, is allosterically inhibited by isoleucine.



Likewise, tryptophan inhibits the enzyme complex that catalyzes the first two steps in the conversion of chorismate into tryptophan.

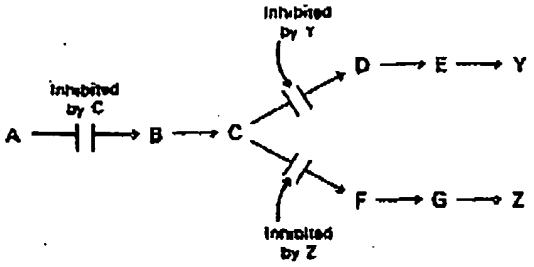
Consider a branched biosynthetic pathway in which Y and Z are the final products.



Suppose that high levels of Y *or* Z completely inhibit the first common step (A → B). Then, high levels of Y would prevent the synthesis of Z even if there were a deficiency of Z. Such a regulatory scheme is obviously not optimal. In fact, several intricate control mechanisms have been found in branched biosynthetic pathways:

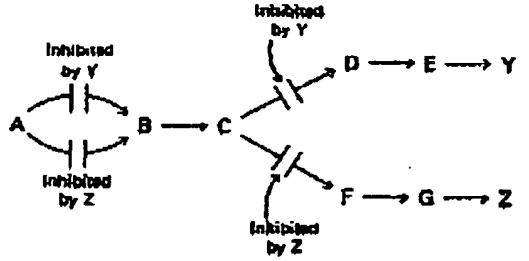
1. *Sequential feedback control.* The first common step (A → B) is not inhibited directly by Y or Z. Rather, these final products inhibit the reactions leading away from the point of branching: Y inhibits the C → D step, and Z inhibits the C → F step. In turn, high levels of

C inhibit the A → B step. Thus, the first common reaction is blocked only if both final products are present in excess.



Sequential feedback control regulates the synthesis of aromatic amino acids in *Bacillus subtilis*. The first divergent steps in the synthesis of phenylalanine, tyrosine, and tryptophan are inhibited by the respective final product. If all three are present in excess, chorismate and prephenate accumulate. These branch-point intermediates in turn inhibit the first common step in the overall pathway, which is the condensation of phosphoenolpyruvate and erythrose 4-phosphate.

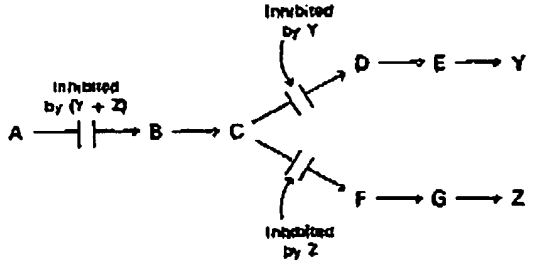
2. *Enzyme multiplicity*. The distinguishing feature of this mechanism is that the first common step (A → B) is catalyzed by two different enzymes. One of them is inhibited by Y, and the other by Z. Thus, both Y and Z must be present at high levels to prevent the conversion of A into B completely. The other aspect of this control scheme is like that in sequential feedback control: Y inhibits the C → D step and Z inhibits the C → F step.



Differential inhibition of multiple enzymes controls a variety of biosynthetic pathways in microorganisms. In *E. coli*, the condensation of phosphoenolpyruvate and erythrose 4-phosphate is catalyzed by three different enzymes. One is inhibited by phenylalanine, another by tyrosine, and the third by tryptophan. Furthermore, there are two different mutases that convert chorismate into prephenate. One of them is inhibited by phenylalanine, the other by tyrosine.

3. *Concerted feedback control*. The first common step (A → B) is inhibited only if high levels of Y and Z are simultaneously present.

A high level of either product alone does not inhibit the A → B step. As in the two control schemes just discussed, Y inhibits the C → D step and Z inhibits the C → F step.



An example of concerted feedback control is the inhibition of aspartyl kinase by threonine and lysine, the final products.

4. *Cumulative feedback control.* The first common step (A → B) is partially inhibited by each of the final products. Each final product acts independently of the others. Suppose that a high level of Y decreased the rate of the A → B step from 100 to 60 sec^{-1} and that Z alone decreased the rate from 100 to 40 sec^{-1}. Then, the rate of the A → B step in the presence of high levels of Y and Z would be 24 sec^{-1} (0.6 × 0.4 × 100 sec^{-1}).

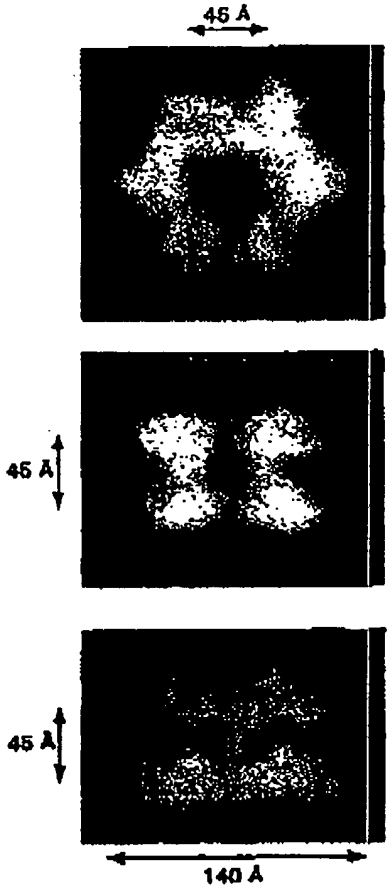


Figure 21-15
Three views of *E. coli* glutamine synthetase obtained by superposing several electron micrographs. The twelve subunits are arranged in two hexagonal rings that face each other. [Courtesy of Dr. Earl Stadtman.]

THE ACTIVITY OF GLUTAMINE SYNTHETASE IS MODULATED BY ADENYLYLATION

The regulation of glutamine synthetase from *E. coli* is a striking example of *cumulative feedback inhibition*. Recall that glutamine is synthesized from glutamate, NH_4^+, and ATP (p. 488). Glutamine synthetase consists of twelve 50-kdal subunits arranged in two hexagonal rings that face each other (Figure 21-15). This enzyme is a key control element in intermediary metabolism because it regulates the flow of nitrogen, as shown by Earl Stadtman and his collaborators. The amide group of glutamine is a source of nitrogen in the biosyntheses of a variety of compounds such as tryptophan, histidine, carbamoyl phosphate, glucosamine 6-phosphate, CTP, and AMP. Glutamine synthetase is cumulatively inhibited by each of these final products of glutamine metabolism, as well as by alanine and glycine. There seem to be specific binding sites for each of these inhibitors. The enzymatic activity of glutamine synthetase is almost completely switched off when all eight final products are bound to the enzyme.

Another important and related feature of glutamine synthetase from *E. coli* is that its activity is altered by *reversible covalent modification* (Figure 21-16). This type of control was previously encountered in the synthesis and degradation of glycogen (p. 368). Phosphorylation activates glycogen phosphorylase and inactivates glycogen synthetase. The activity of glutamine synthetase is regulated in part by the covalent attachment of an *AMP unit* to the hydroxyl group of a

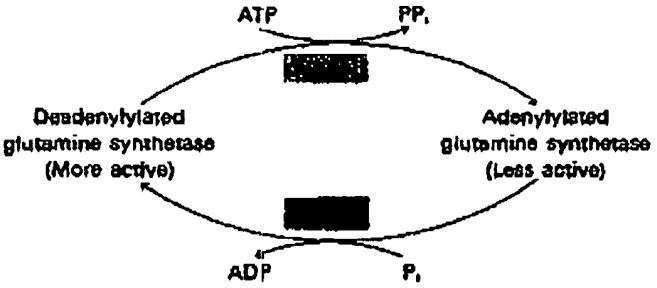
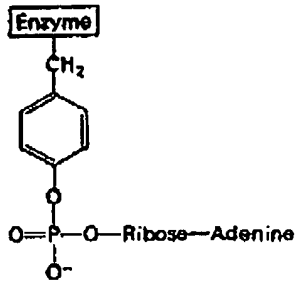


Figure 21-16
Control of the activity of glutamine synthetase by reversible covalent modification. Adenylylation is catalyzed by a complex of adenylyl transferase (AT) and one form of a regulatory protein (P_A). The same enzyme catalyzes deadenylylation when it is complexed with the other form (P_D) of the regulatory protein.



Adenylylated enzyme

specific tyrosine residue in each subunit. *This adenylylated enzyme is more susceptible to cumulative feedback inhibition than the deadenylylated form.* The covalently attached AMP unit can be removed from the adenylylated enzyme by phosphorolysis. An interesting feature of these reactions is that they are catalyzed by the same enzyme, *adenylyl transferase*. What then determines whether it acts to insert or remove an AMP unit? It turns out that the specificity of adenylyl transferase is controlled by a *regulatory protein* (designated P), which can exist in two forms, P_A and P_D. The complex of P_A and adenylyl transferase attaches AMP to glutamine synthetase, which thereby reduces its activity, whereas the complex of P_D and adenylyl transferase removes AMP. This brings us to another level of reversible covalent modification. P_A is converted into P_D by the attachment of uridine monophosphate (UMP), as shown in Figure 21-17. This reaction, which is catalyzed by a *uridyl transferase*, is stimulated by ATP and α-ketoglutarate, whereas it is inhibited by glutamine. In turn, the two UMP units on P_D can be enzymatically removed by hydrolysis.

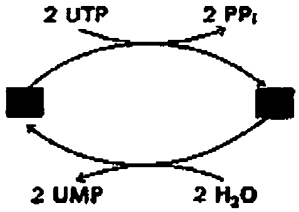


Figure 21-17
A higher level in the regulatory cascade of glutamine synthetase. P_A and P_D, the regulatory proteins that control the specificity of glutamine synthetase, are interconvertible. P_A is converted into P_D by uridylylation, which is reversed by hydrolysis. The enzymes catalyzing these reactions sense the concentrations of metabolic intermediates.

The outcome of this *regulatory cascade* is that adenylylation is inhibited and deadenylylation is stimulated if the supply of activated nitrogen is low. Glutamine synthetase then becomes less susceptible to cumulative feedback inhibition, and the supply of glutamine consequently increases. Why is a cascade used to regulate this enzyme? One advantage of a cascade is that it *amplifies signals*, as in blood clotting (p. 171) and the control of glycogen metabolism (p. 373). Another likely reason is that the *potential for allosteric control is markedly increased because each enzyme in the cascade becomes an independent target for regulation.* The integration of nitrogen metabolism in a cell requires that a large number of input signals be detected and processed. There are limits to what a single protein can accomplish on its own—even a molecule as sentient as glutamine synthetase! The evolution of a cascade provided many more regulatory sites and made possible a finer tuning of the flow of nitrogen in the cell.

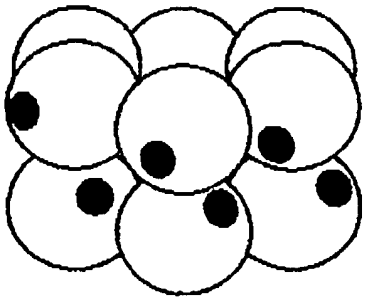


Figure 21-18
A model of glutamine synthetase showing the peripheral site of adenylylation. [After a drawing kindly provided by Dr. David Eisenberg.]

AMINO ACIDS ARE PRECURSORS OF A VARIETY OF BIOMOLECULES

Amino acids are the building blocks of proteins and peptides. They also serve as precursors of many kinds of small molecules that have important biological roles. Let us briefly survey some of the biomolecules that are derived from amino acids (Figure 21-19). *Purines* and

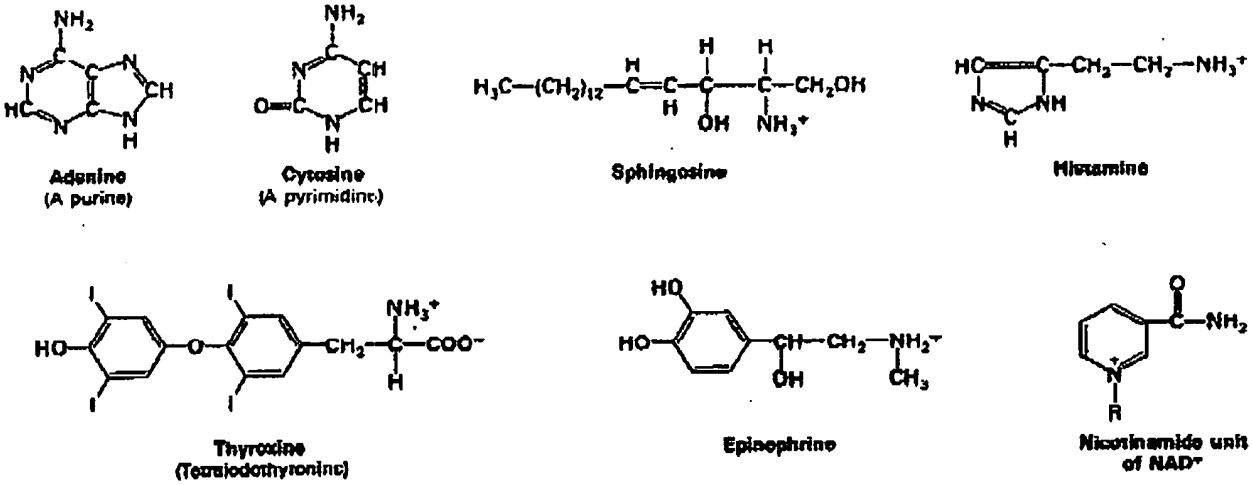


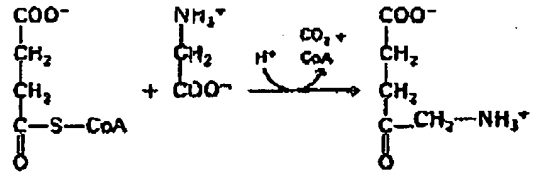
Figure 21-19
Biomolecules derived from amino acids.

pyrimidines are derived in part from amino acids. The biosynthesis of these precursors of DNA, RNA, and numerous coenzymes is discussed in detail in the next chapter. Six of the nine atoms of the purine ring and four of the six atoms of the pyrimidine ring are derived from amino acids. The reactive terminus of *sphingosine*, an intermediate in the synthesis of sphingolipids, comes from serine. *Histamine*, a potent vasodilator, is derived from histidine by decarboxylation. Tyrosine is a precursor of the hormones *thyroxine* (tetraiodothyronine) and *epinephrine* and of *melanin*, a polymeric pigment. The neurotransmitter *5-hydroxytryptamine* (*serotonin*) and the *nicotinamide ring* of NAD^+ are synthesized from tryptophan. Glutamine contributes the amide group of the nicotinamide moiety.

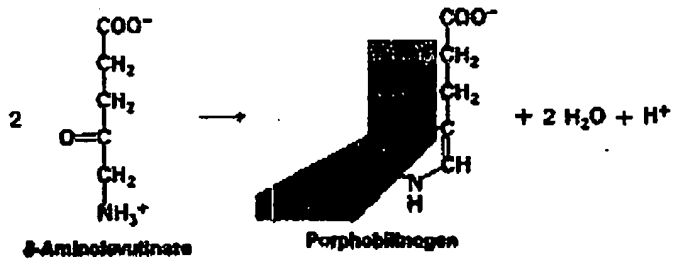
PORPHYRINS ARE SYNTHESIZED FROM GLYCINE AND SUCCINYL COENZYME A

The involvement of an amino acid in the biosynthesis of the porphyrin rings of hemes and chlorophylls was first revealed by isotope-labeling experiments carried out by David Shemin and his

colleagues. In 1945, they showed that the nitrogen atoms of heme were labeled following the feeding of ^{15}N-glycine to human subjects, whereas ^{15}N-glutamate resulted in very little labeling. Using carbon-14, which had just become available, they discovered that in nucleated duck erythrocytes 8 of the carbon atoms of heme are derived from the α-carbon of glycine and none from the carboxyl carbon (Figure 21-20). Subsequent studies demonstrated that the other 26 carbon atoms of heme can arise from acetate. Moreover, the ^{14}C in methyl-labeled acetate emerged in 24 of these 26 carbons, whereas, the ^{14}C in carboxyl-labeled acetate appeared only in the other two. This highly distinctive labeling pattern led Shemin to propose that a heme precursor is formed by the condensation of glycine with an activated succinyl compound. In fact, *the first step in the biosynthesis of porphyrins is the condensation of glycine and succinyl CoA to form δ-aminolevulinate.*



This reaction is catalyzed by δ-aminolevulinate synthetase, a PLP enzyme in mitochondria. As expected, this committed step in the biosynthesis of porphyrins is regulated. Two molecules of δ-aminolevulinate then condense to form *porphobilinogen.* This dehydration reaction is catalyzed by δ-aminolevulinate dehydrase.



Four porphobilinogens condense head-to-tail to form a *linear tetrapyrrole,* which remains bound to the enzyme (Figure 21-21). An ammonium ion is released for each methylene bridge formed. This linear tetrapyrrole cyclizes by losing NH_4^+. The cyclic product is uroporphyrinogen III, which has an asymmetric arrangement of side chains. These reactions require a *synthetase* and a *cosynthetase.* In the presence of synthetase alone, uroporphyrinogen I, the symmetric isomer, is produced. The cosynthetase is essential for isomerizing

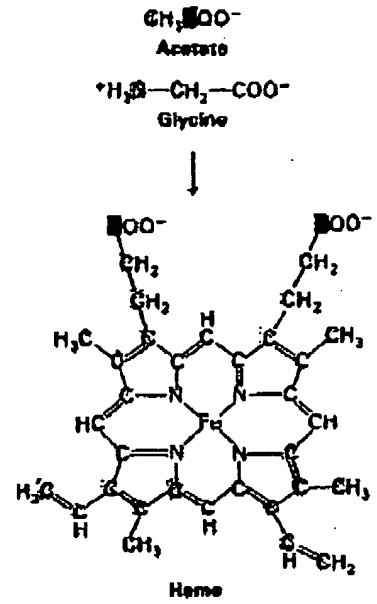


Figure 21-20
Labeling pattern of heme synthesized from glycine and acetate. The nitrogen atoms (blue) arise from the amino group of glycine. The origins of the carbon atoms are: yellow, from the α-carbon of glycine; green, mainly from the methyl carbon of acetate; and red, from the carboxyl carbon of acetate.

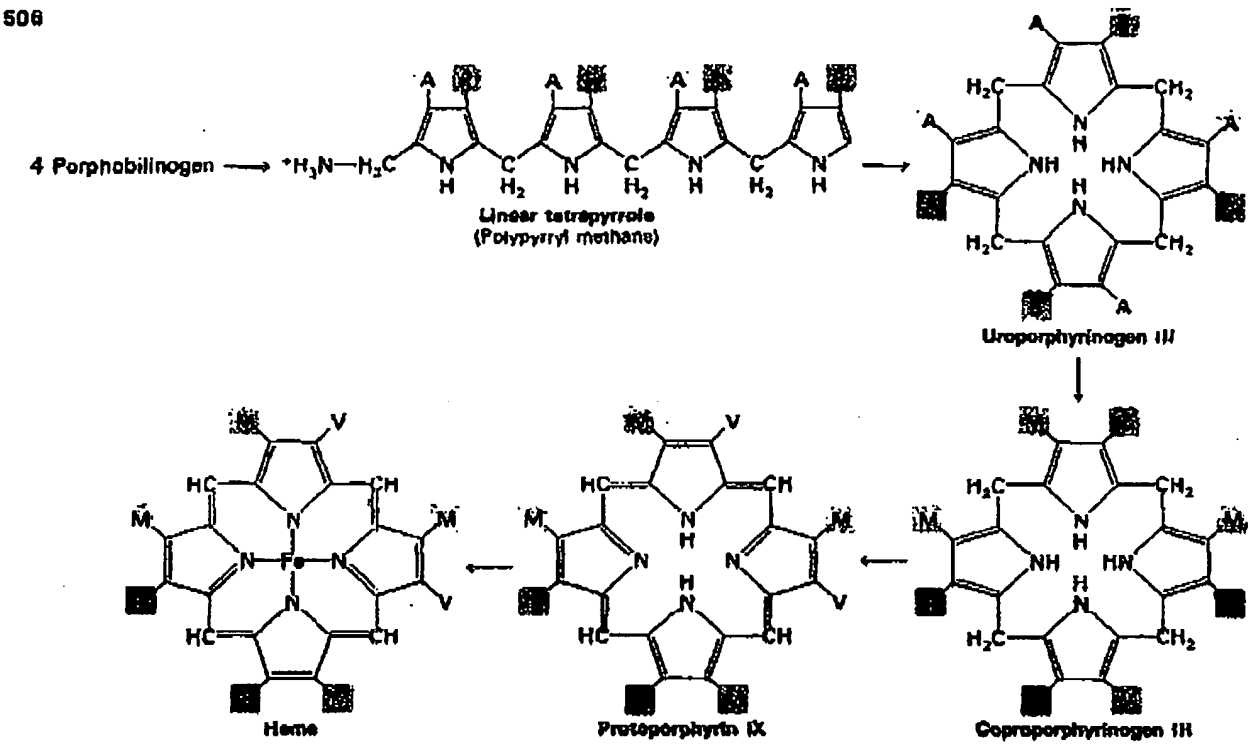


Figure 21-21
Pathway for the synthesis of heme from porphobilinogen. (Abbreviations: A, acetate; M, methyl; P, propionate, V, vinyl.)

one of the pyrrole rings to yield asymmetric uroporphyrinogen III.

The porphyrin skeleton is now formed. Subsequent reactions alter the side chains and the degree of saturation of the porphyrin ring (Figure 21-21). *Coproporphyrinogen III* is formed by decarboxylation of the acetate side chains. Unsaturation of the porphyrin ring and conversion of two of the propionate side chains into vinyl groups yield *protoporphyrin IX*. Chelation of iron finally gives *heme*, the prosthetic group of proteins such as myoglobin, hemoglobin, catalase, peroxidase, and cytochrome *c*. The insertion of the *ferrous* form of iron is catalyzed by *ferrochelatase*. Iron is transported in the plasma by *transferrin*, a protein that binds two ferric ions, and stored in tissues inside molecules of *ferritin*. The large internal cavity (~80 Å diameter) of ferritin can hold as many as 4500 ferric ions.

Several factors that regulate heme biosynthesis in animals have been elucidated. *Delta-aminolevulinate synthetase, the enzyme that catalyzes the committed step in this pathway, is feedback inhibited by heme*, as is δ-aminolevulinate dehydrase and ferrochelatase. Regulation also occurs at the level of enzyme synthesis. *Heme represses the synthesis of δ-aminolevulinate synthetase*. Recent studies suggest that the iron atom itself may be the active regulatory species.

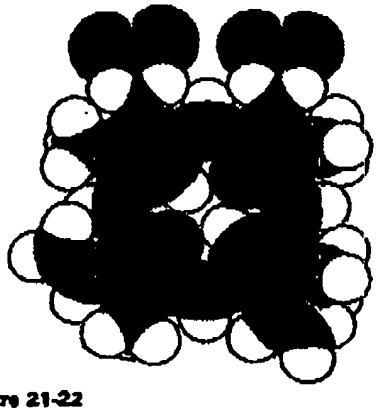
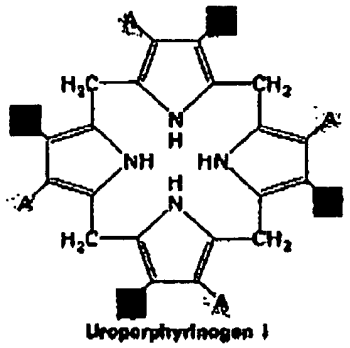


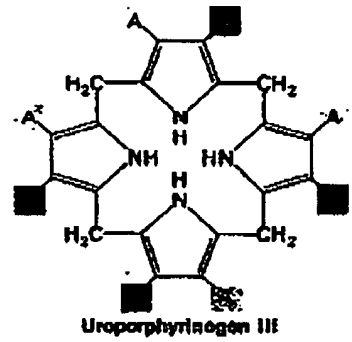
Figure 21-22
Space-filling model of protoporphyrin IX, the immediate precursor of heme.

PORPHYRINS ACCUMULATE IN SOME INHERITED DISORDERS OF PORPHYRIN METABOLISM

Several inherited disorders of porphyrin metabolism are known. In *congenital erythropoietic porphyria*, there is a deficiency of uroporphyrinogen III cosynthetase, the isomerase that yields the asymmetric isomer on cyclization of the linear tetrapyrrole. The synthesis of the required amount of uroporphyrinogen III is accompanied by the formation of very large quantities of uroporphyrinogen I, the symmetric isomer devoid of a physiologic role. Uroporphyrin I, coproporphyrin I, and other symmetric derivatives also accumulate. Erythrocytes are prematurely destroyed in this disease, which is transmitted as an autosomal recessive. The urine of patients having this disease is red because of the excretion of large amounts of uroporphyrin I. Their teeth exhibit a strong red fluorescence under ultraviolet light because of the deposition of porphyrins. Furthermore, their *skin is usually very sensitive to light*.



Uroporphyrinogen I



Uroporphyrinogen III

Acute intermittent porphyria is a quite different disease. The liver, rather than the red cells, is affected, and the skin is not typically photosensitive. The activity of uroporphyrinogen synthetase is decreased in this disorder, and there is a compensatory increase in the level of δ-aminolevulinate synthetase. Consequently, the concentrations of δ-aminolevulinate and porphobilinogen in the liver are increased and so large amounts of these compounds are excreted in the urine. The disease is inherited as an *autosomal dominant*. The striking clinical symptoms are intermittent abdominal pain and neurologic disturbances. As its name implies, the disease is episodic in its clinical expression. Acute attacks are sometimes precipitated by drugs such as barbiturates and estrogens.

BILIVERDIN AND BILIRUBIN ARE INTERMEDIATES IN THE BREAKDOWN OF HEME

The normal human erythrocyte has a life span of about 120 days. Old cells are removed from the circulation and degraded by the spleen. The apoprotein of hemoglobin is hydrolyzed to its constituent amino acids. The first step in the degradation of the heme group to bilirubin (Figure 21-23) is the cleavage of its α-methene bridge to form *biliverdin*, a linear tetrapyrrole. This reaction is catalyzed by *heme oxygenase*. Two aspects of this reaction are noteworthy. First, this enzyme is a *monooxygenase*: O_2 and NADPH are required for the cleavage reaction. Second, a methene bridge carbon is released as *carbon monoxide*. This endogenous production of CO posed a special problem in the evolution of oxygen carriers (p. 55). The central methene bridge of biliverdin is then reduced by biliverdin reductase to form *bilirubin*. Again, the reductant is NADPH. The changing color of a bruise is a highly visible indication of these degradative reactions.

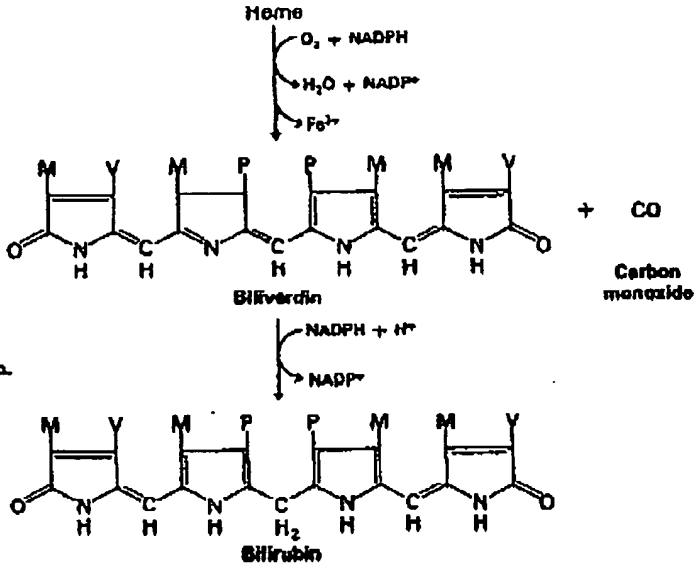
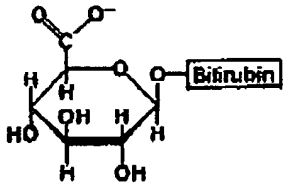


Figure 21-23
Degradation of heme to bilirubin.



A glucuronate unit in bilirubin diglucuronide

Bilirubin complexed to serum albumin is transported to the liver, where it is rendered more soluble by the attachment of sugar residues to its propionate side chains. The solubilizing sugar is *glucuronate*, which differs from glucose in having a COO^- group at C-6 rather than a CH_2OH group. The conjugate of bilirubin and two glucuronates, called *bilirubin diglucuronide*, is secreted into bile. *UDP-glucuronate, derived from the oxidation of UDP-glucose, is the activated intermediate in the synthesis of bilirubin diglucuronide.* Thus, the iron atom of heme is recycled, whereas the organic moiety is converted into a soluble, open-chain form that is excreted.

SUMMARY

Microorganisms use ATP and a powerful reductant to convert N_2 into NH_4^+, which is consumed by higher organisms in the synthesis of amino acids, nucleotides, and other biomolecules. The major points of entry of NH_4^+ into intermediary metabolism are glutamine, glutamate, and carbamoyl phosphate. Humans can synthesize only half of the basic set of twenty amino acids. These amino acids are called nonessential, in contrast with the essential ones, which must be supplied in the diet. The pathways for the synthesis of nonessential amino acids are quite simple. Glutamate dehydrogenase catalyzes the reductive amination of α-ketoglutarate to glutamate. Alanine and aspartate are synthesized by transamination of pyruvate and oxaloacetate, respectively. Glutamine is synthesized from NH_4^+ and glutamate, and asparagine is synthesized similarly.

Proline is derived from glutamate. Serine, formed from 3-phosphoglycerate, is the precursor of glycine and cysteine. Tyrosine is synthesized by the hydroxylation of phenylalanine, an essential amino acid. The pathways for the biosynthesis of essential amino acids are much more complex than for the nonessential ones. Most of these pathways are regulated by feedback inhibition, in which the committed step is allosterically inhibited by the final product. The regulation of glutamine synthetase from *E. coli* provides a striking demonstration of cumulative feedback inhibition and of control by a cascade of reversible covalent modifications.

Tetrahydrofolate, a carrier of activated one-carbon units, plays an important role in amino acid and nucleotide metabolism. This coenzyme carries one-carbon units at three oxidation states, which are interconvertible: most reduced—methyl; intermediate—methylene; most oxidized—formyl, formimino, and methenyl. The major donor of activated methyl groups is *S*-adenosylmethionine, which is synthesized by the transfer of an adenosyl group from ATP to the sulfur atom of methionine. *S*-Adenosylhomocysteine is formed when the activated methyl group is transferred to an acceptor. It is hydrolyzed to adenosine and homocysteine, which is then methylated to methionine to complete the activated methyl cycle.

Amino acids are precursors of a variety of biomolecules. Porphyrins are synthesized from glycine and succinyl CoA, which condense to give δ-aminolevulinate. This intermediate condenses with itself to form porphobilinogen. Four porphobilinogens combine to form a linear tetrapyrrole, which cyclizes to form uroporphyrinogen III. Oxidation and side-chain modifications lead to the synthesis of protoporphyrin IX, which acquires an iron atom to form heme. Delta-aminolevulinate synthetase, the enzyme that catalyzes the committed step in this pathway, is feedback inhibited by heme.

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PROBLEMS

1. Write a balanced equation for the synthesis of alanine from glucose.
2. What are the intermediates in the flow of nitrogen from N_2 to heme?
3. What derivative of folate is a reactant in each of the following conversions?
   (a) Glycine → serine.
   (b) Histidine → glutamate.
   (c) Homocysteine → methionine.
4. In the reaction catalyzed by glutamine synthetase, an oxygen atom is transferred from the side chain of glutamate to orthophosphate, as shown by ^{18}O-labeling studies. Propose an interpretation for this finding.
5. Isovaleric acidemia is an inherited disorder of leucine metabolism caused by a deficiency of isovaleryl-CoA dehydrogenase. Many infants having this disease die in the first month of life. It has recently been reported that the administration of large amounts of glycine to two infants with this disease led to marked clinical improvement. What is the rationale for the use of glycine therapy?
6. Nitrogenase catalyzes the formation of HD in the presence of D_2, a strong reductant, and ATP. Furthermore, H-D exchange depends on the presence of N_2. Propose an intermediate in N_2 fixation that would account for these observations.
7. Blue-green algae form *heterocysts* when deprived of ammonia and nitrate. They lack nuclei and are attached to adjacent vegetative cells. Heterocysts have photosystem I activity but are entirely devoid of photosystem II activity. What is their role?

CHAPTER 22

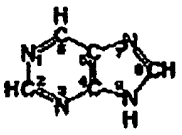
BIOSYNTHESIS OF NUCLEOTIDES

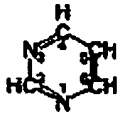
This chapter deals with the biosynthesis of nucleotides. These compounds participate in nearly all biochemical processes:

1. They are the *activated precursors of DNA and RNA*.
2. Nucleotide derivatives are *activated intermediates in many biosyntheses*. For example, UDP-glucose and CDP-diacylglycerol are precursors of glycogen and phosphoglycerides, respectively.
3. ATP, an adenine nucleotide, is the *universal currency of energy* in biological systems.
4. Adenine nucleotides are *components of three major coenzymes*: NAD^+, FAD, and CoA.
5. Nucleotides are *metabolic regulators*. Cyclic AMP is a ubiquitous mediator of the action of many hormones. Covalent modifications introduced by ATP alter the activities of some enzymes, as exemplified by the phosphorylation of glycogen synthetase and the adenylylation of glutamine synthetase.

NOMENCLATURE OF BASES, NUCLEOSIDES, AND NUCLEOTIDES

A nucleotide consists of a nitrogenous base, a sugar, and one or more phosphate groups. The nitrogenous base is a *purine* or *pyrimidine* derivative. The two major purines are *adenine* and *guanine*, and


Purine


Pyrimidine

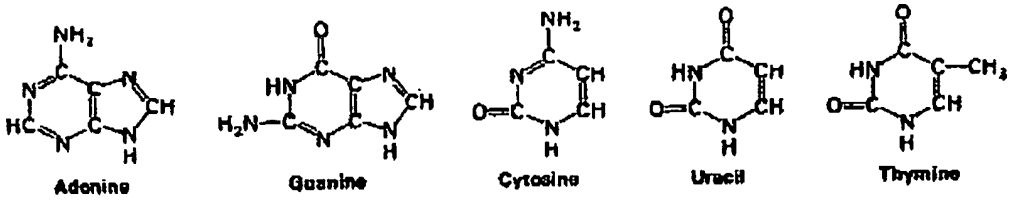
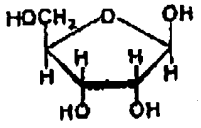
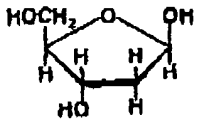


Figure 22-1
Structures of the major purines and pyrimidines.





the three major pyrimidines are *cytosine, uracil,* and *thymine* (Figure 22-1).

A *nucleoside* consists of a purine or pyrimidine base linked to a pentose. The pentose is D-*ribose* or *2-deoxy*-D-*ribose*. In a nucleoside, the glycosidic C-1 carbon atom of the pentose is bonded to N-1 of the pyrimidine or N-9 of the purine base. The configuration of this *N*-glycosidic linkage is β in all naturally occurring nucleosides.

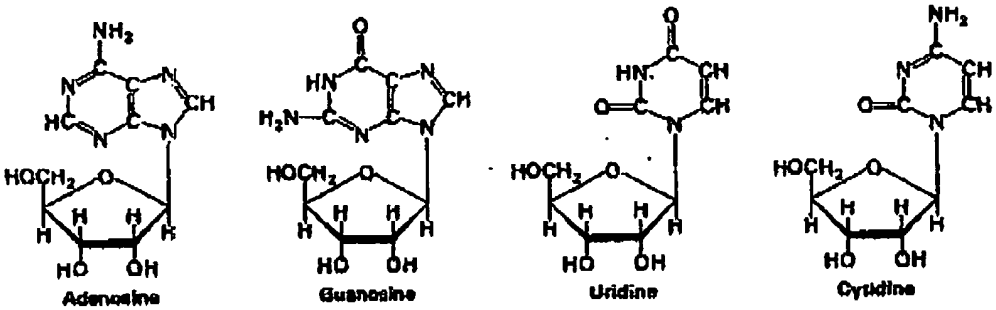


Figure 22-2
Structures of the major ribonucleosides.

In a ribonucleoside, the pentose is ribose, whereas in a deoxyribonucleoside it is deoxyribose. The major ribonucleosides are *adenosine, guanosine, uridine,* and *cytidine*. The major deoxyribonucleosides are *deoxyadenosine, deoxyguanosine, deoxythymidine,* and *deoxycytidine*.

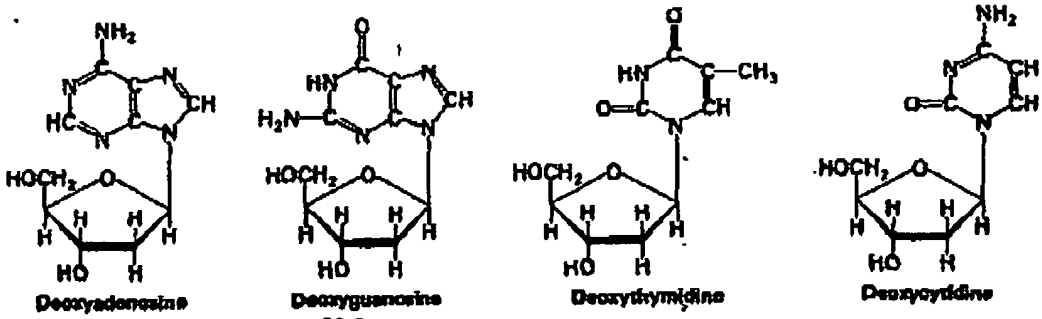


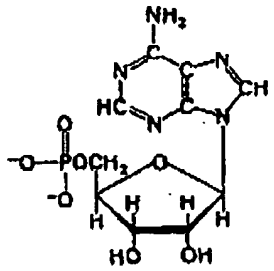
Figure 22-3
Structures of the major deoxyribonucleosides.

Note that uracil is replaced by thymine, its methylated analog, in the deoxy series.

A *nucleotide* is a phosphate ester of a nucleoside. At least one of the hydroxyl groups of the pentose moiety of a nucleotide is esterified. The most common site of esterification is the hydroxyl group attached to C-5 of the pentose. Such a compound is called a *nucleoside 5′-phosphate* or a *5′-nucleotide*. A primed number designates an atom of the pentose, whereas an unprimed number designates an atom of the purine or pyrimidine ring. The type of pentose is denoted by the prefix in the terms *5′-ribonucleotide* and *5′-deoxyribonucleotide*.

The nucleotide derived by esterification of the 5′-hydroxyl group of adenosine is *adenosine 5′-phosphate*, which is often called *adenylate*. The name adenylic acid is sometimes used, but adenylate is preferred because the phosphate group is ionized at physiological pH. The standard abbreviation for this compound is AMP (for adenosine monophosphate). The common names of the other major 5′-ribonucleotides are *guanylate* (GMP), *uridylate* (UMP), and *cytidylate* (CMP). The major 5′-deoxyribonucleotides are called *deoxyadenylate* (dAMP), *deoxyguanylate* (dGMP), *deoxythymidylate* (dTMP), and *deoxycytidylate* (dCMP). The first letter in their abbreviations is a *d* to denote that they are 2′-deoxyribonucleotides.

In a *nucleoside 5′-diphosphate*, a diphosphate group is esterified to the 5′-hydroxyl of the pentose, whereas in a *nucleoside 5′-triphosphate*, a triphosphate group is linked to this hydroxyl. Thus, the adenine ribonucleotide series is called adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), and adenosine 5′-triphosphate (ATP). The corresponding deoxyribonucleotides are deoxyadenosine 5′-monophosphate (dAMP), deoxyadenosine 5′-diphosphate (dADP), and deoxyadenosine 5′-triphosphate (dATP). The nomenclature of bases, nucleosides, and nucleotides is summarized in Table 22-1.


Adenosine 5′-phosphate (AMP)

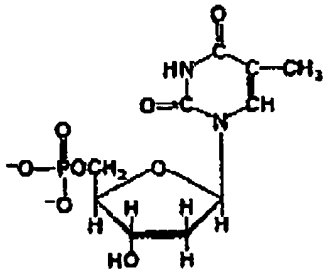

Thymidine 5′-phosphate (TMP)

Table 22-1
Nomenclature of bases, nucleosides, and nucleotides

Base	*Ribonucleoside*	*Ribonucleotide (5′-monophosphate)*
Adenine (A)	Adenosine	Adenylate (AMP)
Guanine (G)	Guanosine	Guanylate (GMP)
Uracil (U)	Uridine	Uridylate (UMP)
Cytosine (C)	Cytidine	Cytidylate (CMP)
Base	*Deoxyribonucleoside*	*Deoxyribonucleotide (5′-monophosphate)*
Adenine (A)	Deoxyadenosine	Deoxyadenylate (dAMP)
Guanine (G)	Deoxyguanosine	Deoxyguanylate (dGMP)
Thymine (T)	Deoxythymidine	Deoxythymidylate (dTMP)
Cytosine (C)	Deoxycytidine	Deoxycytidylate (dCMP)

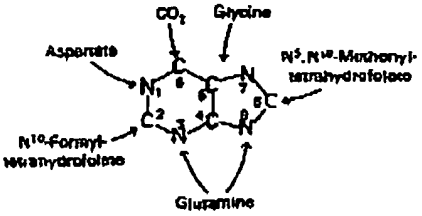


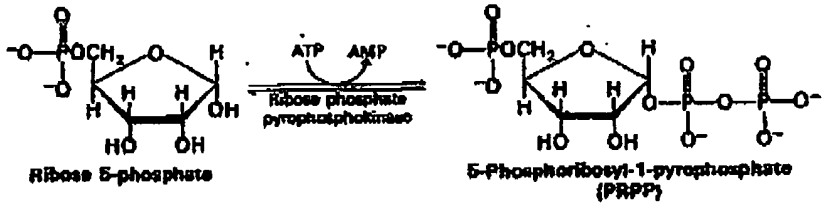
Figure 22-4
Origins of the atoms in the purine ring.

THE PURINE RING IS SYNTHESIZED FROM AMINO ACIDS, TETRAHYDROFOLATE DERIVATIVES, AND CO_2

The purine ring in purine nucleotides is assembled from a variety of precursors (Figure 22-4). *Glycine* provides C-4, C-5, and N-7. The N-1 atom comes from *aspartate*. The other two nitrogen atoms, N-3 and N-9, come from the amide group of the side chain of *glutamine*. Activated derivatives of *tetrahydrofolate* furnish C-2 and C-8, whereas CO_2 is the source of C-6.

PRPP IS THE DONOR OF THE RIBOSE PHOSPHATE MOIETY OF NUCLEOTIDES

The pathway of purine biosynthesis was elucidated in the 1950s by John Buchanan, G. Robert Greenberg, and others. The ribose phosphate portion of purine and pyrimidine nucleotides comes from *5-phosphoribosyl-1-pyrophosphate* (PRPP), a key intermediate in the biosynthesis of histidine and tryptophan. PRPP is synthesized from ATP and ribose 5-phosphate, which is primarily formed by the pentose phosphate pathway (p. 393). The pyrophosphate group is transferred from ATP to C-1 of ribose 5-phosphate. PRPP has an α configuration.



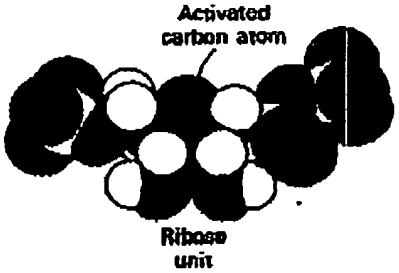
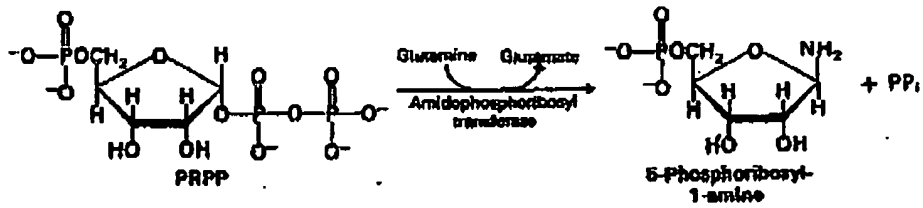


Figure 22-5
Space-filling model of 5-phosphoribosyl-1-pyrophosphate (PRPP), the activated donor of the sugar unit in the biosynthesis of nucleotides.

THE PURINE RING IS ATTACHED TO RIBOSE PHOSPHATE DURING ITS ASSEMBLY

The committed step in the de novo synthesis of purine nucleotides is the formation of *5-phosphoribosylamine* from PRPP and glutamine. The amino group from the side chain of glutamine displaces the pyrophosphate group attached to C-1 of PRPP. The configuration at C-1 is inverted from α to β in this reaction. The resulting C–N glycosidic bond has the β configuration that is characteristic of naturally occurring nucleotides. This reaction is driven forward by the hydrolysis of pyrophosphate.



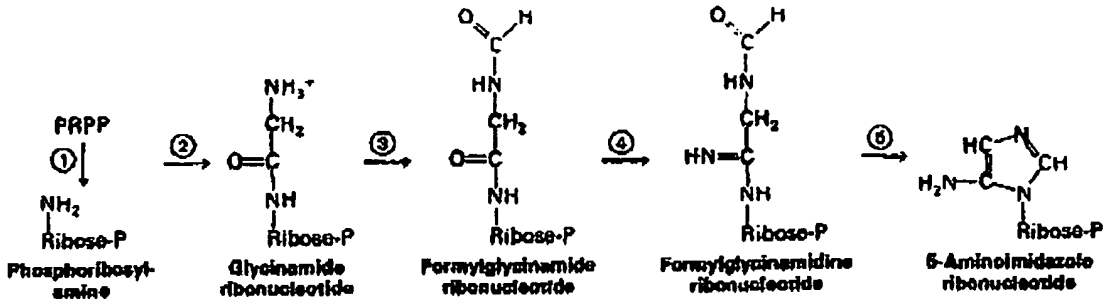


Figure 22-6
First stage of purine biosynthesis: formation of 5-aminoimidazole ribonucleotide from PRPP. The essence of these reactions is (1) displacement of PP_i by the side-chain amino group of glutamine, (2) addition of glycine, (3) formylation by methenyltetrahydrofolate, (4) transfer of a nitrogen atom from glutamine, and (5) dehydration and ring closure.

Glycine joins phosphoribosylamine to yield *glycinamide ribonucleotide* (Figure 22-6). An ATP is consumed in the formation of an amide bond between the carboxyl group of glycine and the amino group of phosphoribosylamine. The α-amino terminus of the glycine residue is then formylated by methenyltetrahydrofolate to give *α-N-formylglycinamide ribonucleotide*. The keto group in this compound is converted into an amidine group. The nitrogen atom is donated by the side chain of glutamine in a reaction that consumes an ATP. *Formylglycinamidine ribonucleotide then undergoes ring closure to form 5-aminoimidazole ribonucleotide*. This intermediate contains the complete five-membered ring of the purine skeleton.

The next phase in the synthesis of the purine skeleton, the formation of a six-membered ring, starts at this point (Figure 22-7). Three

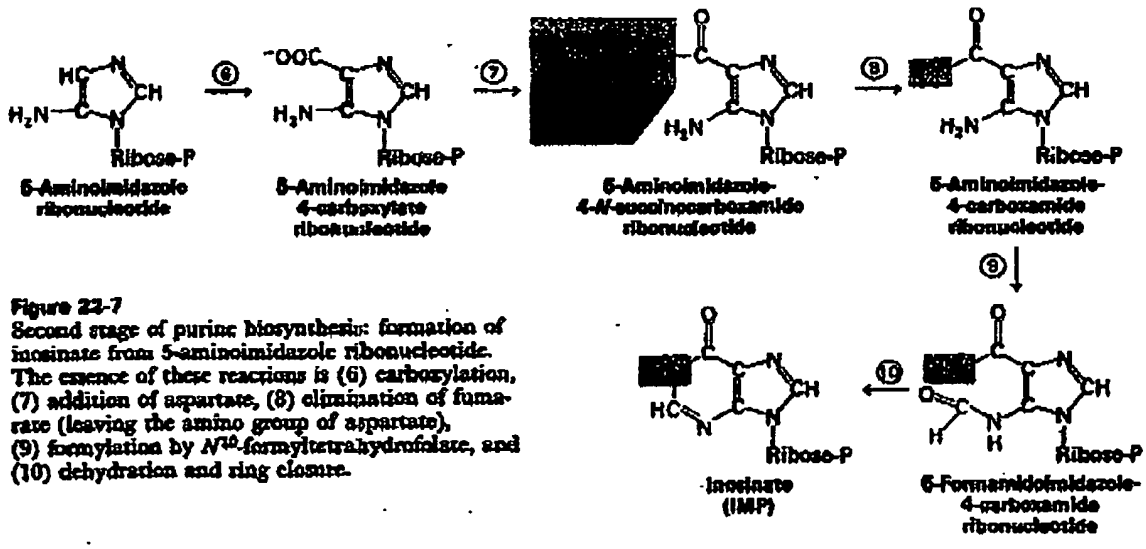


Figure 22-7
Second stage of purine biosynthesis: formation of inosinate from 5-aminoimidazole ribonucleotide. The essence of these reactions is (6) carboxylation, (7) addition of aspartate, (8) elimination of fumarate (leaving the amino group of aspartate), (9) formylation by N^{10}-formyltetrahydrofolate, and (10) dehydration and ring closure.

of the six atoms of this ring are already present in aminoimidazole ribonucleotide. The other three come from CO_2, aspartate, and formyltetrahydrofolate. The next carbon atom in the six-membered ring is introduced by the carboxylation of aminoimidazole ribonucleotide, yielding *5-aminoimidazole-4-carboxylate ribonucleotide*.

The amino group of aspartate then reacts with the carboxyl group of this intermediate to form *5-aminoimidazole-4-N-succinocarboxamide ribonucleotide*. An ATP is consumed in the formation of this amide bond. The carbon skeleton of the aspartate moiety comes off as fumarate in the next reaction, which yields *5-aminoimidazole-4-carboxamide ribonucleotide*. Note that the result of these two reactions is the conversion of a carboxylate into an amide. Thus, *aspartate contributes only its nitrogen atom to the purine ring*. The final atom of the purine ring is contributed by N^{10}-formyltetrahydrofolate. The resulting *5-formamidoimidazole-4-carboxamide ribonucleotide* undergoes dehydration and ring closure to form *inosinate* (IMP), which contains a complete purine ring. The purine-base part of inosinate is called *hypoxanthine*.

AMP AND GMP ARE FORMED FROM IMP

Inosinate is the precursor of AMP and GMP (Figure 22-8). *Adenylate* is synthesized from inosinate by the insertion of an amino group at C-6 in place of the carbonyl oxygen. Aspartate again contributes its amino group by addition of this amino acid followed by elimination of fumarate. GTP is the donor of a high-energy phosphate bond in the synthesis of *adenylosuccinate* from inosinate and aspartate. The removal of fumarate from adenylosuccinate and from 5-aminoimidazole-4-*N*-succinocarboxamide ribonucleotide is catalyzed by the same enzyme.

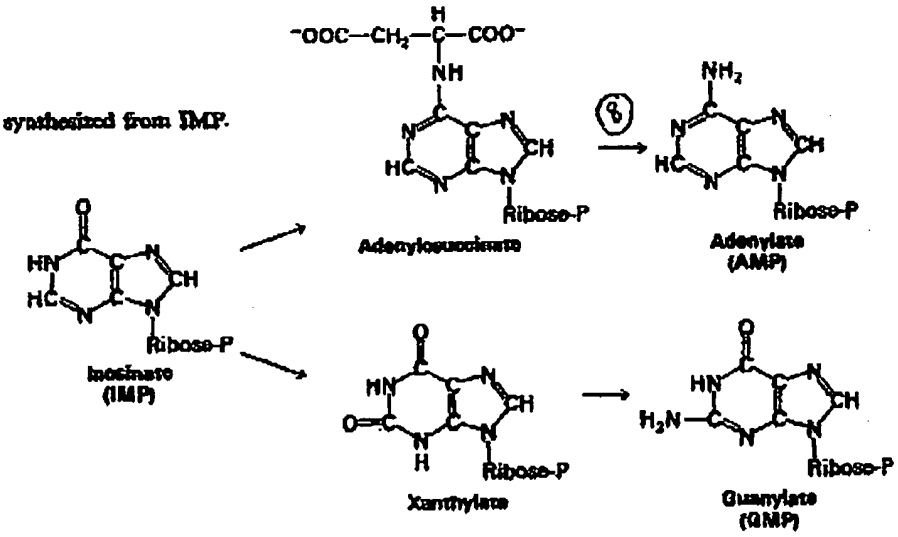


Figure 22-8
AMP and GMP are synthesized from IMP.

Guanylate (GMP) is synthesized by the oxidation of inosinate, followed by the insertion of an amino group at C-2. NAD^+ is the hydrogen acceptor in the oxidation of inosinate to xanthylate (XMP). The amino group in the side chain of glutamine is then transferred to xanthylate. Two high-energy phosphate bonds are consumed in this reaction, because ATP is cleaved into AMP and PP_i, which is subsequently hydrolyzed.

In the conversion of inosinate into adenylate and into guanylate, a carbonyl oxygen atom is replaced by an amino group. A similar change occurs in the synthesis of formylglycinamide ribonucleotide from its amide precursor (step 4 on p. 515), in the formation of CTP from UTP (p. 522), and in the conversion of citrulline into arginine in the urea cycle (p. 419). *The common mechanistic theme of these reactions is the conversion of the carbonyl oxygen into a derivative that can be readily displaced by an amino group.* The tautomeric form of the carbonyl group reacts with ATP (or GTP) to form a phosphoryl ester, which is nucleophilically attacked by an amine (Figure 22-9). Inor-

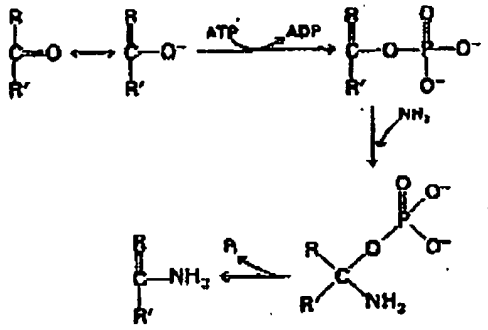


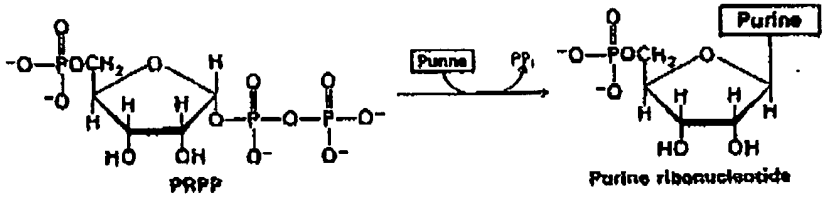
Figure 22-9
Reaction mechanism for the replacement of a carbonyl oxygen by an amino group.

ganic phosphate is then expelled from this tetrahedral adduct to complete the reaction. The attacking amine can be NH_3, the side-chain amide group of glutamine, or the α-amino group of aspartate. The leaving group in this class of reactions can be P_i, PP_i, or the AMP moiety. For example, PP_i is displaced by the amino group of glutamine in the synthesis of 5-phosphoribosyl-1-amine from PRPP (p. 514).

PURINE BASES CAN BE RECYCLED BY SALVAGE REACTIONS THAT UTILIZE PRPP

Free purine bases are formed by the hydrolytic degradation of nucleic acids and nucleotides. Purine nucleotides can be synthesized from these preformed bases by a *salvage reaction*, which is simpler and much less costly than the reactions of the *de novo pathway* discussed above. In the salvage reaction, the ribose phosphate moiety

of PRPP is transferred to the purine to form the corresponding nucleotide:



There are two salvage enzymes with different specificities. *Adenine phosphoribosyl transferase* catalyzes the formation of adenylate:

$$\text{Adenine} + \text{PRPP} \longrightarrow \text{adenylate} + \text{PP}_i$$

whereas *hypoxanthine-guanine phosphoribosyl transferase* catalyzes the formation of inosinate and guanylate:

$$\text{Hypoxanthine} + \text{PRPP} \longrightarrow \text{inosinate} + \text{PP}_i$$
$$\text{Guanine} + \text{PRPP} \longrightarrow \text{guanylate} + \text{PP}_i$$

The versatile and efficient use of the purine ring is also evident in the biosynthesis of histidine. The six-membered portion of the purine ring of ATP contributes part of the imidazole ring of histidine (p. 499). The rest of the purine skeleton is not discarded. Rather, it is conserved in 5-aminoimidazole-4-carboxamide ribonucleotide, an intermediate in the de novo pathway of purine biosynthesis.

AMP AND GMP ARE FEEDBACK INHIBITORS OF PURINE NUCLEOTIDE BIOSYNTHESIS

The synthesis of purine nucleotides is controlled by feedback inhibition at several sites (Figure 22-10).

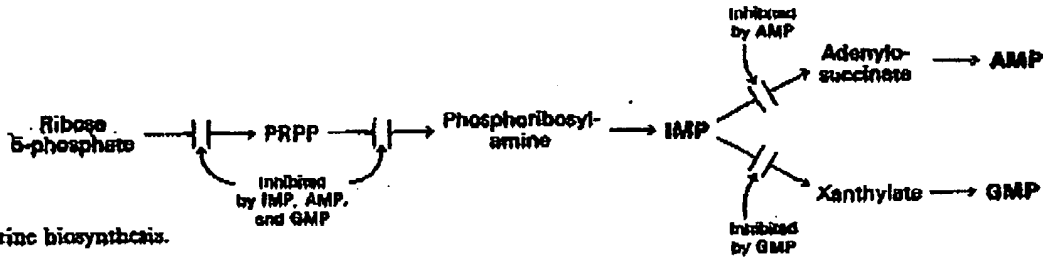


Figure 22-10
Control of purine biosynthesis.

1. Feedback inhibition of *5-phosphoribosyl-1-pyrophosphate synthetase* by purine nucleotides regulates the level of PRPP. This synthetase is inhibited by AMP, GMP, and IMP.

2. The committed step in purine nucleotide biosynthesis is the conversion of PRPP into phosphoribosylamine by transfer of the

side-chain amino group of glutamine. *Glutamine PRPP amidotransferase is feedback inhibited by many purine ribonucleotides.* It is noteworthy that AMP and GMP, the final products of the pathway, are synergistic in inhibiting this enzyme.

3. Inosinate is the branching point in the synthesis of AMP and GMP. *The reactions leading away from inosinate are sites of feedback inhibition.* AMP inhibits the conversion of inosinate into adenylosuccinate, its immediate precursor. Similarly, GMP inhibits the conversion of inosinate into xanthylate, its immediate precursor.

4. GTP is a substrate in the synthesis of AMP, whereas ATP is a substrate in the synthesis of GMP. This *reciprocal substrate relation* tends to balance the synthesis of adenine and guanine ribonucleotides.

THE PYRIMIDINE RING IS SYNTHESIZED FROM CARBAMOYL PHOSPHATE AND ASPARTATE

The pyrimidine ring is assembled first and then linked to ribose phosphate to form a pyrimidine nucleotide, in contrast with the reaction sequence in the de novo synthesis of purine nucleotides. PRPP is the donor of ribose phosphate in the synthesis of pyrimidine nucleotides, as well as of purine nucleotides. The precursors of the pyrimidine ring are carbamoyl phosphate and aspartate (Figure 22-11).

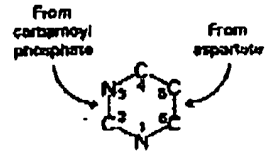
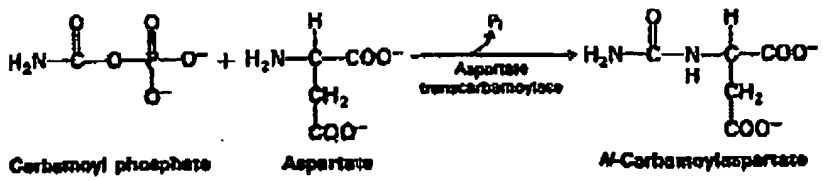


Figure 22-11
Origins of the atoms in the pyrimidine ring. C-2 and N-3 come from carbamoyl phosphate, whereas the other atoms of the ring come from aspartate.

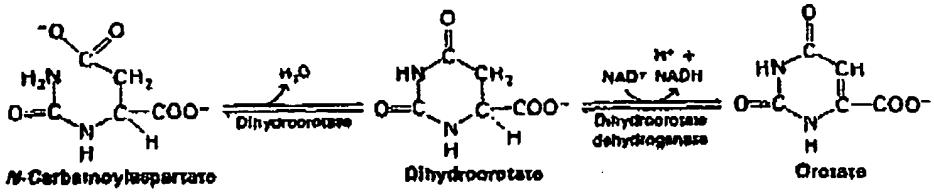
The synthesis of pyrimidines starts with the formation of *carbamoyl phosphate*, which is also an intermediate in the synthesis of urea (p. 412). The synthesis of this activated carbamoyl donor is compartmentalized in eucaryotes. Carbamoyl phosphate consumed in the synthesis of pyrimidines is formed in the cytosol, whereas that used in the synthesis of urea is formed in mitochondria (p. 414). There are two distinct carbamoyl phosphate synthetases. Another noteworthy difference is that glutamine rather than NH_4^+ is the nitrogen donor in the cytosol synthesis of carbamoyl phosphate.

$$\text{Glutamine} + 2\,\text{ATP} + HCO_3^- \longrightarrow \text{carbamoyl phosphate} + 2\,\text{ADP} + P_i + \text{glutamate}$$

The committed step in the biosynthesis of pyrimidines is the formation of N-*carbamoylaspartate* from aspartate and carbamoyl phosphate. This carbamoylation is catalyzed by *aspartate transcarbamoylase*, an especially interesting regulatory enzyme (see p. 522).

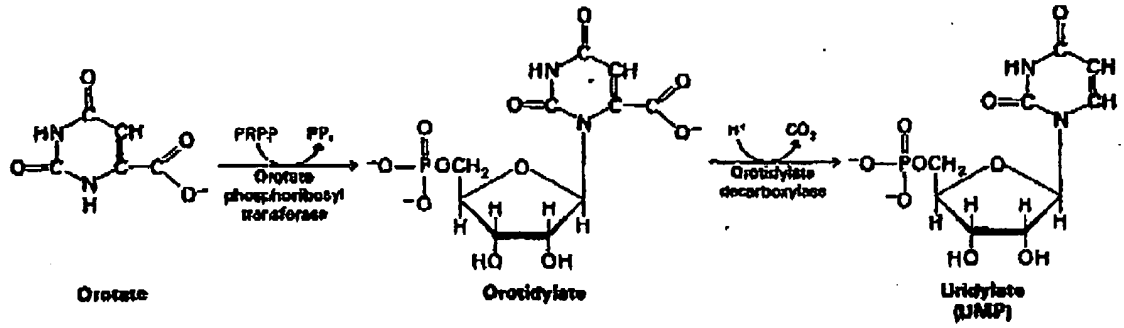


The pyrimidine ring is formed in the next reaction, in which carbamoylaspartate cyclizes with loss of water to yield *dihydroorotate*. *Orotate* is then formed by dehydrogenation of dihydroorotate.



OROTATE ACQUIRES A RIBOSE PHOSPHATE MOIETY FROM PRPP

The next step in the synthesis of pyrimidine nucleotides is the *acquisition of a ribose phosphate group*. Orotate (a free pyrimidine) reacts with PRPP to form *orotidylate* (a pyrimidine nucleotide). This reaction, which is catalyzed by orotidylate pyrophosphorylase, is driven forward by the hydrolysis of pyrophosphate. Orotidylate is then decarboxylated to yield *uridylate* (UMP), a major pyrimidine nucleotide.



A SINGLE POLYPEPTIDE CHAIN CONTAINS THE FIRST THREE ENZYMES OF PYRIMIDINE BIOSYNTHESIS

In *E. coli*, the six enzymes that synthesize UMP from simple precursors do not appear to be associated. In contrast, in higher organisms, several of these enzymes form a multienzyme complex. Large amounts of this complex were obtained by treating cultured mammalian cells with *N-(phosphonacetyl)-L-aspartate* (PALA), a potent inhibitor of aspartate transcarbamoylase (ATCase). PALA binds tightly to ATCase ($K_i = 10^{-8}$ M) because it has some of the structural features of the transition state in catalysis (Figure 22-12). The surviving cells overcame the inhibitory effect of PALA by synthesizing 100-fold more ATCase than do normal cells. The levels of carbamoyl phosphate synthetase and of dihydroorotase were also

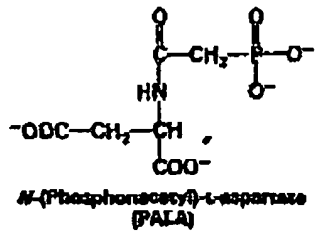


Figure 22-12
Structure of PALA, a potent inhibitor of ATCase.

elevated 100-fold, whereas there was little change in the amounts of the enzymes catalyzing the subsequent steps in pyrimidine biosynthesis. These observations led to the finding that *carbamoyl phosphate synthetase, aspartate transcarbamoylase, and dihydroorotase are covalently joined on a single 200-kdal polypeptide chain.* Orotate phosphoribosyltransferase and orotidylate decarboxylase, the enzymes catalyzing the last two steps in pyrimidine biosynthesis, form another complex. They, too, may be covalently joined. Recall that the fatty acid synthetase complex in yeast consists of two kinds of polypeptide chains, each containing several enzymes (p. 400). *Covalent linkage of functionally related enzymes may be quite general in eucaryotes.* Such an arrangement would facilitate the assembly of a multienzyme complex. Another likely advantage of having several enzymes on a single polypeptide chain is that they would be synthesized in equimolar amounts.

NUCLEOSIDE MONO-, DI-, AND TRIPHOSPHATES ARE INTERCONVERTIBLE

The active forms of nucleotides in biosyntheses and energy conversions are the diphosphates and triphosphates. Nucleoside monophosphates are converted by specific *nucleoside monophosphate kinases* that utilize ATP as the phosphoryl donor. For example, UMP is phosphorylated by *UMP kinase.*

$$\text{UMP} + \text{ATP} \rightleftharpoons \text{UDP} + \text{ADP}$$

AMP, ADP, and ATP are interconverted by *adenylate kinase* (also called myokinase). The equilibrium constants of these reactions are close to 1.

$$\text{AMP} + \text{ATP} \rightleftharpoons \text{ADP} + \text{ADP}$$

Nucleoside diphosphates and triphosphates are interconverted by *nucleoside diphosphate kinase,* an enzyme that has broad specificity, in contrast with the monophosphate kinases. In the following equation, X and Y can be any of several ribonucleosides or deoxyribonucleosides.

$$\text{XDP} + \text{YTP} \rightleftharpoons \text{XTP} + \text{YDP}$$

For example,

$$\text{UDP} + \text{ATP} \rightleftharpoons \text{UTP} + \text{ADP}$$

CTP IS FORMED BY AMINATION OF UTP

Cytidine triphosphate (CTP) is derived from uridine triphosphate (UTP), the other major pyrimidine ribonucleotide. The carbonyl oxygen at C-4 is replaced by an amino group. In mammals, the side chain of glutamine is the amino donor, whereas NH_4^+ is used in this

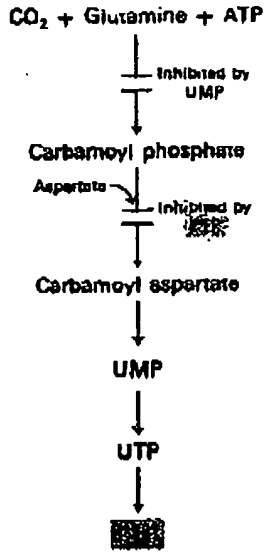


Figure 22-13
Control of pyrimidine biosynthesis.

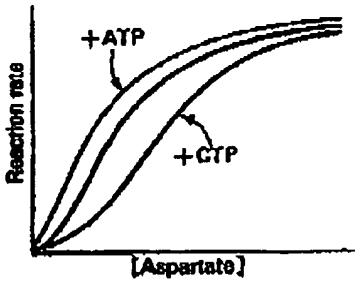
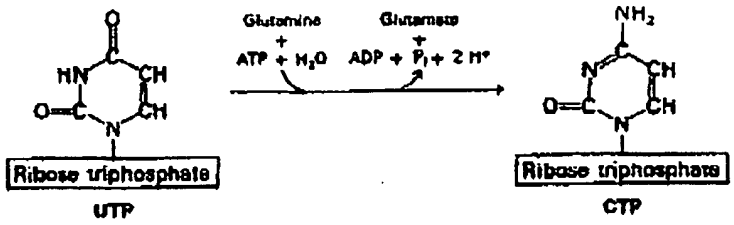


Figure 22-14
Allosteric effects in aspartate transcarbamoylase. ATP is an activator, whereas CTP is an inhibitor. [After J. C. Gerhart, *Curr. Top. Cell Regul.* 2(1970):275.]

reaction in *E. coli*. An ATP is consumed in both amination reactions.



PYRIMIDINE NUCLEOTIDE BIOSYNTHESIS IS REGULATED BY FEEDBACK INHIBITION

The committed step in pyrimidine nucleotide biosynthesis in *E. coli* is the formation of *N*-carbamoylaspartate from aspartate and carbamoyl phosphate. *Aspartate transcarbamoylase, the enzyme that catalyzes this reaction, is feedback inhibited by CTP, the final product in the pathway.* A second control site is *carbamoyl phosphate synthetase,* which is feedback inhibited by UMP (Figure 22-13).

The allosteric properties of ATCase have been intensively investigated by John Gerhart and Howard Schachman. *The binding of carbamoyl phosphate and aspartate is cooperative,* as reflected in the sigmoidal dependence of the reaction velocity on substrate concentration (Figure 22-14). *CTP inhibits the enzyme by decreasing its affinity for substrates* without affecting its V_{max}. The extent of inhibition exerted by CTP, which may reach 90%, depends on the concentrations of the substrates. In contrast, *ATP is an activator of ATCase.* The affinity of the enzyme for its substrates is enhanced by ATP, whereas V_{max} is unaffected. Furthermore, the binding of ATP and CTP to the regulatory site of ATCase is competitive. High levels of ATP displace CTP from the enzyme so that it cannot exert its inhibitory effect.

The biological significance of the activation of ATCase by ATP is twofold. First, *it tends to equalize the rates of formation of purine and pyrimidine nucleotides.* Comparable quantities of these two types of nucleotides are needed for the synthesis of nucleic acids. Second, *activation by ATP signals its availability as a substrate* for some of the reactions of pyrimidine nucleotide biosynthesis, such as the synthesis of carbamoyl phosphate and the phosphorylations of UMP to UTP.

ASPARTATE TRANSCARBAMOYLASE CONSISTS OF SEPARABLE CATALYTIC AND REGULATORY SUBUNITS

The regulatory properties of ATCase vanish when the enzyme is treated with mercurials such as *p*-hydroxymercuribenzoate. ATP

and CTP no longer have any effect on catalytic activity. Furthermore, the binding of substrates becomes noncooperative. However, the modified enzyme has full catalytic activity. This loss of regulatory properties with retention of enzymatic activity is called *desensitization*.

The desensitization of ATCase by mercurials is accompanied by its *dissociation into two kinds of subunits*, as shown by ultracentrifuge studies (Figure 22-15). The sedimentation coefficient of the native enzyme is 11.6S, whereas that of the dissociated subunits is 2.8S and 5.8S. These subunits can be readily separated by ion-exchange chromatography because they differ markedly in charge or by centrifugation in a sucrose density gradient because they differ in size. *p*-Hydroxymercuribenzoate, the dissociating agent, can be removed after the subunits are separated. The larger of the subunits, called the *catalytic subunit*, is catalytically active. However, the activity of the isolated catalytic subunit is not affected by ATP and CTP. The smaller of the subunits, called the *regulatory subunit*, is devoid of catalytic activity but contains specific binding sites for CTP and ATP. The catalytic subunit consists of three 34-kdal polypeptide chains, whereas the regulatory subunit is made up of two 17-kdal polypeptide chains.

The catalytic and regulatory subunits combine rapidly when they are mixed. The resulting complex has the same structure, R_6C_6, as that of the native enzyme.

$$3\,R_2 + 2\,C_3 \longrightarrow R_6C_6$$

Furthermore, *the reconstituted enzyme has the same allosteric properties as those of the native enzyme*.

X-ray crystallographic studies of ATCase are in progress in William Lipscomb's laboratory. An electron-density map at 3.0-Å resolution shows that the two catalytic trimers (C_3) are above and below an equatorial belt of three regulatory dimers (R_2) (Figure 22-16). A distinctive feature of the molecule is that it contains a large central cavity, which is accessible through several channels. It is interesting to note that the allosteric sites for CTP are far from the catalytic sites.

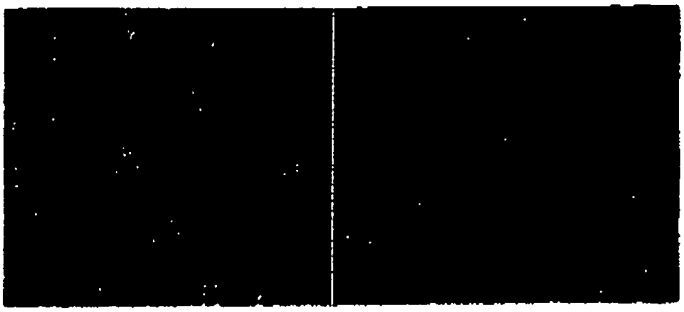
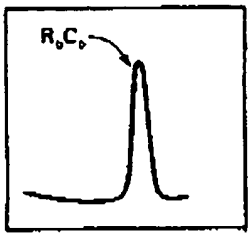
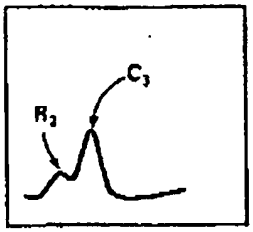


Figure 22-17
Electron micrograph of aspartate transcarbamoylase. [Courtesy of Dr. Robley C. Williams.]



A



B

Figure 22-15
Sedimentation velocity patterns of (A) native ATCase and (B) the enzyme dissociated by a mercurial into regulatory and catalytic subunits. [After J. C. Gerhart and H. K. Schachman. *Biochemistry* 4(1965):1054.]

Figure 22-16
Arrangement of the catalytic (C, shown in blue) and regulatory (R, shown in red) subunits of aspartate transcarbamoylase. [After a drawing kindly provided by Dr. William Lipscomb.]

DEOXYRIBONUCLEOTIDES ARE SYNTHESIZED BY REDUCTION OF RIBONUCLEOSIDE DIPHOSPHATES

We turn now to the synthesis of deoxyribonucleotides. These precursors of DNA are formed by the reduction of ribonucleotides. The 2′-hydroxyl group on the ribose moiety is replaced by a hydrogen atom. In *E. coli* and in mammals, the substrates in this reaction are ribonucleoside *di*phosphates. The overall stoichiometry is

$$\text{Ribonucleoside diphosphate} + \text{NADPH} + \text{H}^+ \longrightarrow \text{deoxyribonucleoside diphosphate} + \text{NADP}^+ + \text{H}_2\text{O}$$

The actual reaction mechanism is more complex than implied by this equation. Peter Reichard has shown that in *E. coli* the electrons from NADPH are transferred to the substrate through a series of sulfhydryl groups. *Ribonucleotide reductase* (also called ribonucleoside diphosphate reductase) catalyzes the final stage, which has the stoichiometry

$$\text{Ribonucleoside diphosphate} + \text{R}\begin{matrix}\text{SH}\\\text{SH}\end{matrix} \longrightarrow \text{deoxyribonucleoside diphosphate} + \text{R}\begin{matrix}\text{S}\\\text{S}\end{matrix} + \text{H}_2\text{O}$$

This enzyme consists of two subunits, B1 (a 160-kdal dimer) and B2 (a 78-kdal dimer). The B1 subunit contains the binding sites for ribonucleotide substrates and for allosteric effectors. In addition, B1 contains sulfhydryls that serve as the immediate electron donors in the reduction of the ribose unit. B2, an iron-sulfur protein, participates in catalysis by forming an unusual free radical on the aromatic ring of a tyrosine residue. The B1 and B2 subunits together form the active sites of the enzyme (Figure 22-18).

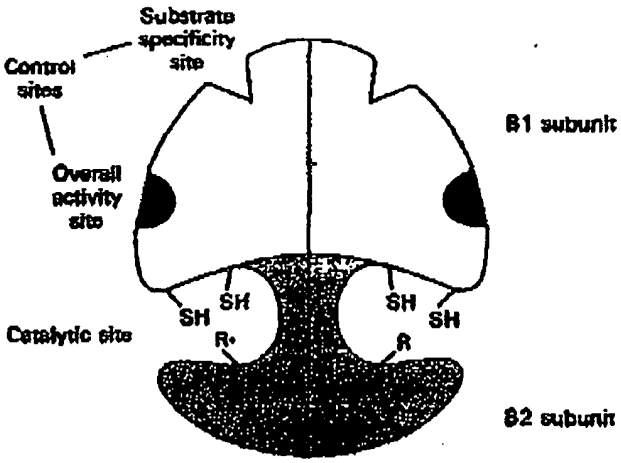
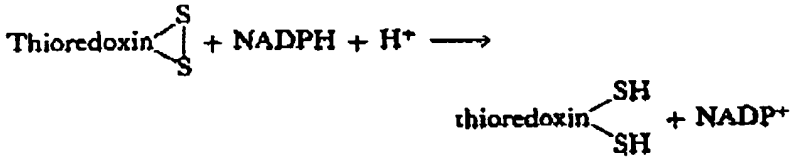


Figure 22-18
Model of ribonucleotide reductase from *E. coli*. [After L. Thelander and P. Reichard. *Ann. Rev. Biochem.* 48(1979):136. ©1979 by Annual Reviews Inc.]

How are electrons transferred from NADPH to the sulfhydryl groups at the catalytic site of ribonucleotide reductase? One carrier of reducing power is *thioredoxin*, a 12-kdal protein with two cysteine residues in close proximity (Figure 22-19). These sulfhydryls are oxidized to a disulfide in the reaction catalyzed by ribonucleotide reductase. In turn, reduced thioredoxin is regenerated by the reaction of NADPH with oxidized thioredoxin. This reaction is catalyzed by *thioredoxin reductase*, a flavoprotein.



It was thought for some time that thioredoxin is the only carrier of reducing power to ribonucleotide reductase. However, a mutant of *E. coli* totally devoid of thioredoxin was found to form deoxyribonucleotides. This surprising observation led to the isolation of a second carrier system. The electron donor in this mutant proved to be *glutathione*, a cysteine-containing tripeptide. As discussed previously (p. 345), *glutathione reductase* catalyzes the reduction of oxidized glutathione (the disulfide form) by NADPH. In addition, *glutaredoxin*, a new protein, is needed to transfer the reducing power of glutathione to ribonucleotide reductase (Figure 22-20). The relative contributions of the thioredoxin and glutaredoxin systems in ribonucleotide reduction by normal cells are not yet known.

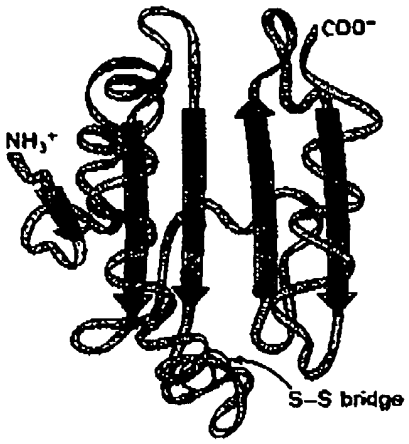


Figure 22-19
Schematic diagram of the main-chain conformation of oxidized thioredoxin from *E. coli*. The reactive disulfide is shown in yellow. [After a drawing kindly provided by Dr. Carl-Ivar Brändén.]

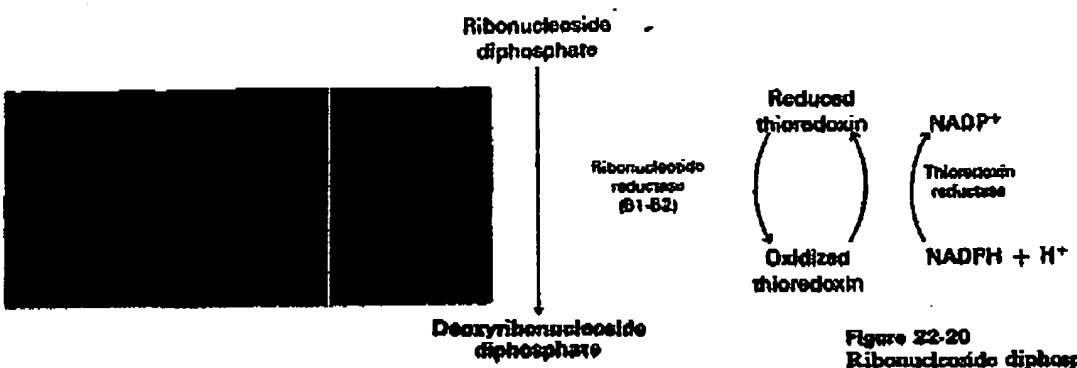


Figure 22-20
Ribonucleoside diphosphates are reduced to deoxyribonucleoside diphosphates by ribonucleotide reductase. Electrons are transferred from NADPH through a series of sulfhydryls. The thioredoxin system (shown in yellow) and the glutaredoxin system (shown in green) can serve as sources of reducing power.

The reduction of ribonucleotide diphosphates is precisely controlled by allosteric interactions. The B1 subunit of ribonucleotide reductase contains two types of allosteric sites: one of them controls the *overall activity* of the enzyme, whereas the other regulates *substrate specificity*. The overall catalytic activity of ribonucleotide reductase is diminished by the binding of dATP, which signals an abundance of deoxyribonucleotides. This feedback inhibition is reversed by the binding of ATP. The binding of dATP or ATP to the substrate-

specificity control sites enhances the reduction of UDP and CDP, the pyrimidine nucleotides. The reduction of GDP is promoted by the binding of dTTP, which also inhibits the further reduction of pyrimidine ribonucleotides. The subsequent increase in the level of dGTP leads to a stimulation of ADP reduction. It is evident that ribonucleotide reductase has a variety of conformational states, each with different catalytic properties. This complex pattern of regulation provides the appropriate supply of the four deoxyribonucleotides needed for the synthesis of DNA.

DEOXYTHYMIDYLATE IS FORMED BY METHYLATION OF DEOXYURIDYLATE

Uracil is not a component of DNA. Rather, DNA contains *thymine*, the methylated analog of uracil. This finishing touch occurs at the level of the deoxyribonucleoside monophosphate: deoxyuridylate (dUMP) is methylated to deoxythymidylate (dTMP) by *thymidylate synthetase*. The methyl donor in this reaction is a tetrahydrofolate derivative rather than *S*-adenosylmethionine. Specifically, the methyl carbon comes from N^5,N^{10}-methylenetetrahydrofolate. Note that the methyl group inserted into deoxyuridylate is more reduced than the methylene group in this tetrahydrofolate derivative. What is the source of electrons for this reduction? The two electrons come from the tetrahydrofolate moiety itself in the form of a hydride ion (H^-), which is removed from the ring. This hydrogen becomes part of the methyl group of dTMP. In this reaction, tetrahydrofolate is oxidized to dihydrofolate. Thus N^5,N^{10}-methylenetetrahydrofolate serves both as an *electron donor* and as a *one-carbon* donor in the methylation reaction (Figure 22-21).

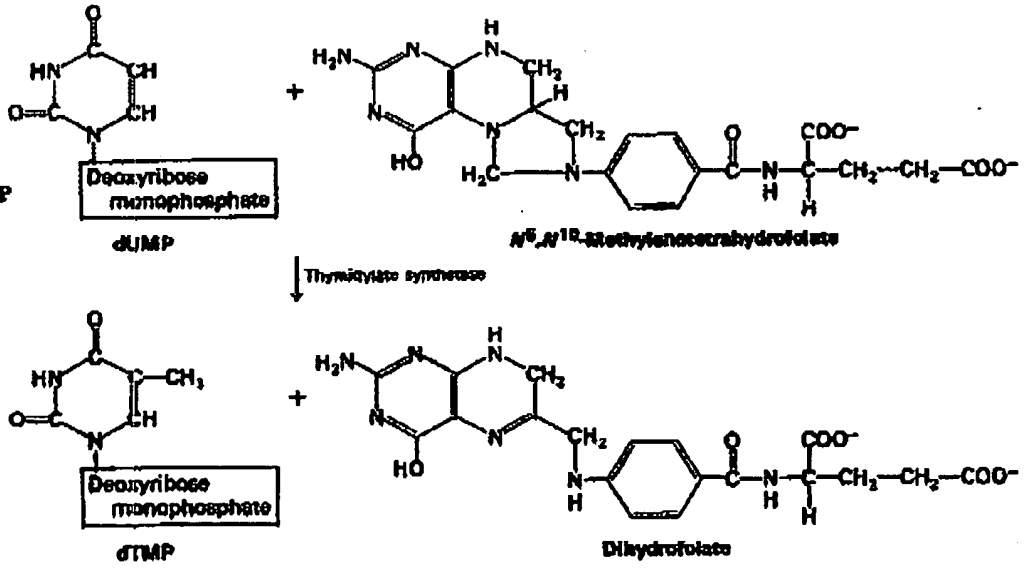


Figure 22-21
Synthesis of dTMP from dUMP.

Recall that one-carbon transfers occur at the level of tetrahydrofolate rather than dihydrofolate. Hence, tetrahydrofolate must be regenerated. This is accomplished by *dihydrofolate reductase*, utilizing NADPH as the reductant.

$$\text{Dihydrofolate} + \text{NADPH} + \text{H}^+ \longrightarrow \text{tetrahydrofolate} + \text{NADP}^+$$

SEVERAL ANTICANCER DRUGS BLOCK THE SYNTHESIS OF DEOXYTHYMIDYLATE

Rapidly dividing cells require an abundant supply of deoxythymidylate for the synthesis of DNA. The vulnerability of these cells to the inhibition of dTMP synthesis has been exploited in cancer chemotherapy (Figure 22-22). Thymidylate synthetase and dihydrofolate reductase are choice target enzymes. *Fluorouracil* (or fluorodeoxyuridine), a clinically useful anticancer drug, is converted in vivo into *fluorodeoxyuridylate* (F-dUMP). *This analog of dUMP irreversibly inhibits thymidylate synthetase after acting as a normal substrate through part of the catalytic cycle.* First, a sulfhydryl group of the enzyme adds to C-6 of the bound F-dUMP. Methylenetetrahydrofolate then adds to C-5 of this intermediate. In the case of dUMP, a hydride ion is subsequently shifted to the methylene group of the folate, and a proton is taken away from C-5 of the bound nucleotide. However,

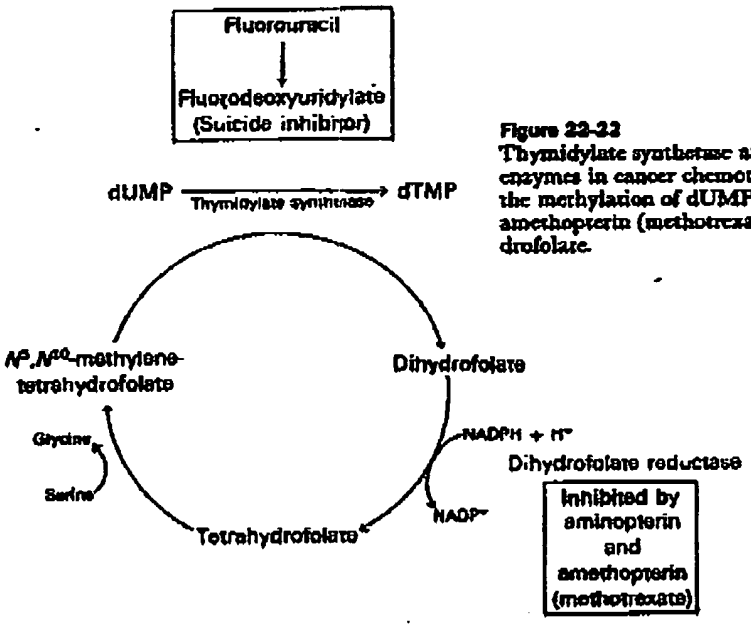


Figure 22-22
Thymidylate synthetase and dihydrofolate reductase are target enzymes in cancer chemotherapy. Fluorodeoxyuridylate inhibits the methylation of dUMP. The folate analogs aminopterin and amethopterin (methotrexate) block the regeneration of tetrahydrofolate.

for F-dUMP, F^+ cannot be abstracted by the enzyme and so catalysis is blocked at the stage of the covalent complex formed by F-dUMP, methylenetetrahydrofolate, and the sulfhydryl group of the enzyme (Figure 22-23).

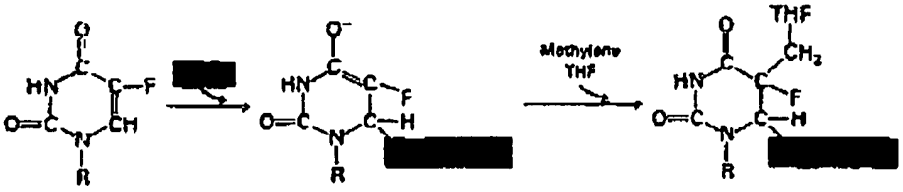
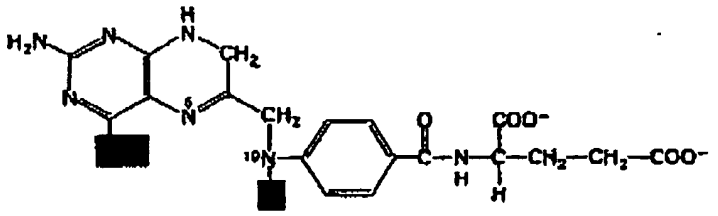


Figure 22-23
Thymidylate synthetase is irreversibly inhibited by fluorodeoxyuridylate (F-dUMP). This analog forms a covalent complex with both a sulfhydryl residue of the enzyme (shown in blue) and methylenetetrahydrofolate (shown in yellow).

The synthesis of dTMP can also be blocked by inhibiting the regeneration of tetrahydrofolate (Figure 22-22). Analogs of dihydrofolate, such as *aminopterin* and *amethopterin* (*methotrexate*) are potent competitive inhibitors ($K_i < 10^{-9}$ M) of dihydrofolate reductase (Figure 22-24). Amethopterin is a valuable drug in the treatment of acute leukemia and choriocarcinoma.



Structure of aminopterin (R = H) and amethopterin (R = CH_3)

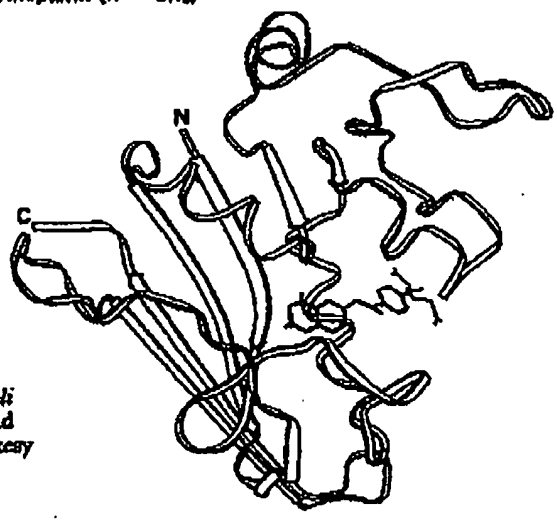
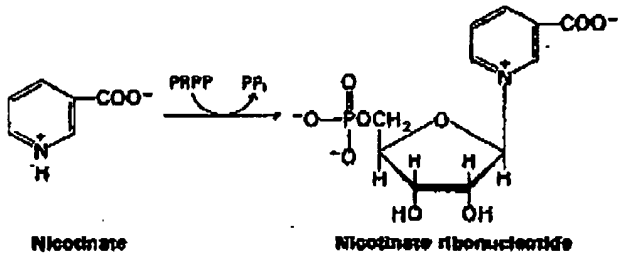


Figure 22-24
Three-dimensional structure of *E. coli* dihydrofolate reductase with a bound amethopterin (methotrexate). [Courtesy of Dr. Joseph Kraut.]

The *biosynthesis of nicotinamide adenine dinucleotide* (NAD^+) starts with the formation of *nicotinate ribonucleotide* from nicotinate and PRPP. *Nicotinate* (also called *niacin*) is derived from tryptophan. Humans can synthesize the required amount of nicotinate if the supply of tryptophan in the diet is adequate. However, an exogenous supply of nicotinate is required if the dietary intake of tryptophan is low. *Pellagra* is a deficiency disease caused by a dietary insufficiency of tryptophan and nicotinate.



An AMP moiety is transferred from ATP to nicotinate ribonucleotide to form *deamido-NAD+*. The final step is the transfer of the amide group of glutamine to the nicotinate carboxyl group to form NAD^+ (Figure 22-25). $NADP^+$ is derived from NAD^+ by phospho-

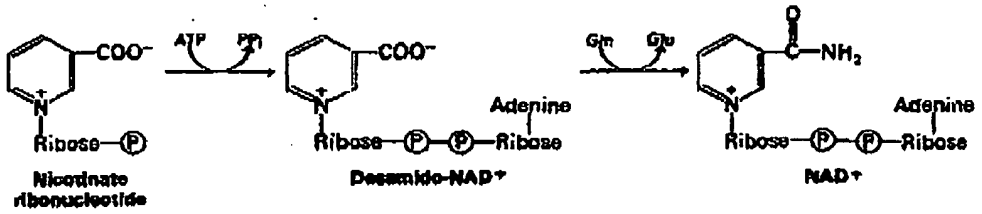


Figure 22-25
Synthesis of NAD^+ from nicotinate ribonucleotide.

rylation of the 2′-hydroxyl group of the adenine ribose moiety. This transfer of a phosphoryl group is catalyzed by NAD^+ kinase.

Flavin adenine dinucleotide (FAD) is synthesized from riboflavin and two molecules of ATP. Riboflavin is phosphorylated by ATP to give *riboflavin 5′-phosphate* (also called *flavin mononucleotide*). FAD is then formed by the transfer of an AMP moiety from a second molecule of ATP to riboflavin 5′-phosphate.

$$\text{Riboflavin} + \text{ATP} \longrightarrow \text{riboflavin 5′-phosphate} + \text{ADP}$$

$$\text{Riboflavin 5′-phosphate} + \text{ATP} \rightleftharpoons \text{flavin adenine dinucleotide} + PP_i$$

The *synthesis of coenzyme A* (CoA) in animals starts with the phosphorylation of *pantothenate* (Figure 22-26). Pantothenate is required in the diet of animals, whereas it is synthesized by plants and microorganisms. A peptide bond is formed between the carboxyl group of 4′-phosphopantothenate and the amino group of cysteine. The carboxyl group of the cysteine moiety is lost, which results in *4′-phosphopantotheine*. The AMP moiety of ATP is then transferred to this intermediate to form *dephosphocoenzyme A*. Finally, phosphorylation of its 3′-hydroxyl group yields coenzyme A.

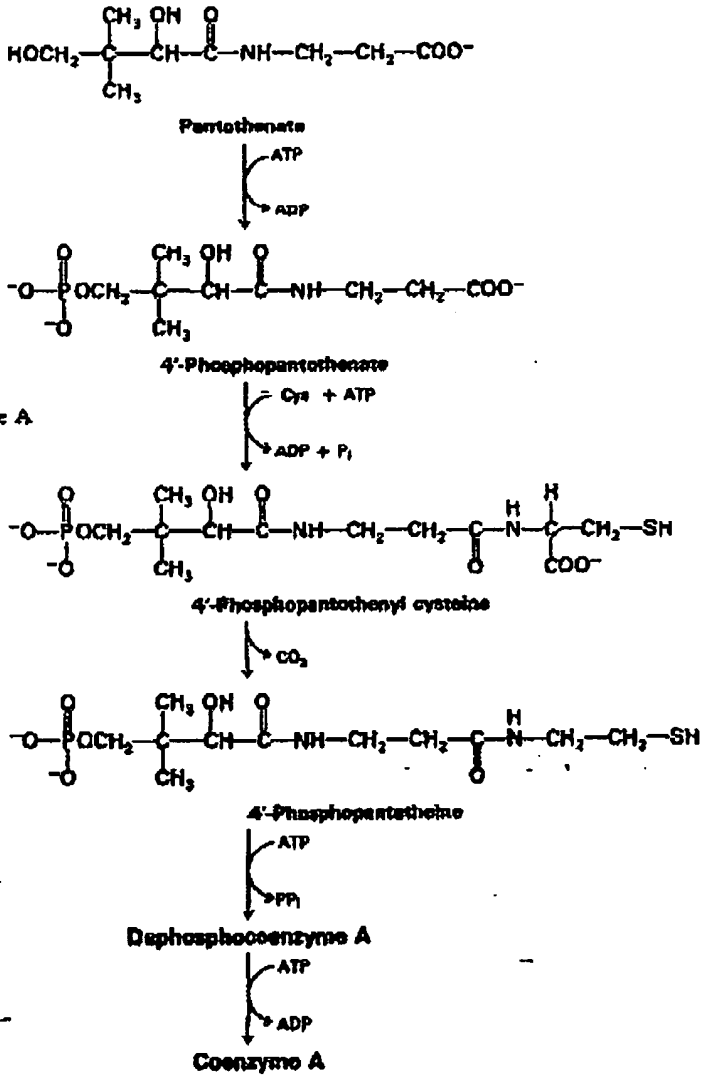


Figure 22-26
Synthesis of coenzyme A from pantothenate.

A common feature of the biosyntheses of NAD^+, FAD, and CoA is the *transfer of the AMP moiety of ATP to the phosphate group of a phosphorylated intermediate.* The pyrophosphate formed in this reaction is hydrolyzed to orthophosphate. This is a recurring motif in biochemistry: *biosynthetic reactions are frequently driven by the hydrolysis of the released pyrophosphate.*

PURINES ARE DEGRADED TO URATE IN HUMANS

The nucleotides of a cell undergo continuous turnover. Nucleotides are hydrolytically degraded to nucleosides by *nucleotidases.* Phosphorolytic cleavage of nucleosides to free bases and ribose 1-phosphate (or deoxyribose 1-phosphate) is catalyzed by *nucleoside phosphorylases.* Ribose 1-phosphate is isomerized by *phosphoribomutase* to ribose 5-phosphate, a substrate in the synthesis of PRPP. Some of the bases are reused to form nucleotides by salvage pathways.

The pathway for the degradation of AMP (Figure 22-27) includes an additional step. AMP is deaminated to IMP by *adenylate*

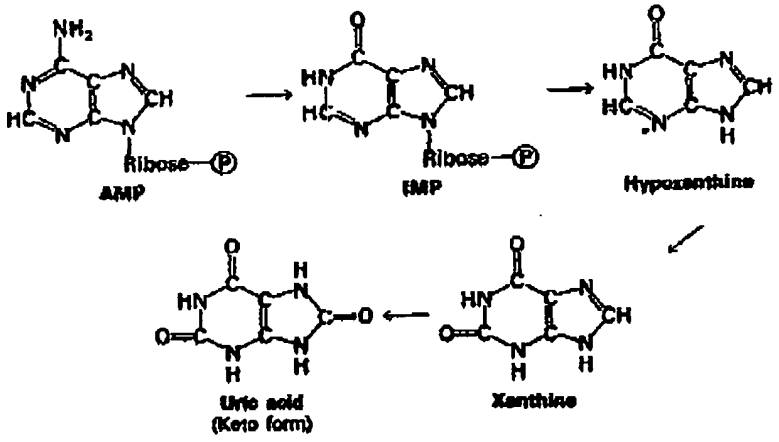


Figure 22-27
Degradation of AMP to uric acid.

deaminase. The subsequent reactions leading to the free base hypoxanthine follow the general pattern. *Xanthine oxidase,* a molybdenum and iron-containing flavoprotein, oxidizes hypoxanthine to *xanthine* and then to *urate.* Molecular oxygen, the oxidant in both reactions, is reduced to H_2O_2, which is decomposed to H_2O and O_2 by catalase. Xanthine is also an intermediate in the formation of urate from guanine. *In humans, urate is the final product of purine degradation and is excreted in the urine.*

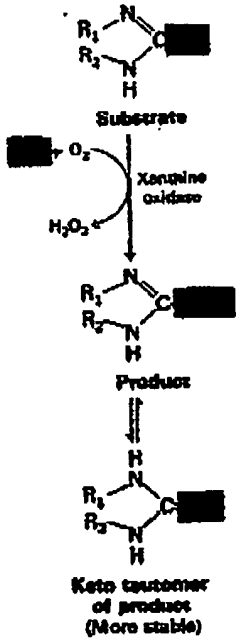


Figure 22-28
Oxidation reaction catalyzed by xanthine oxidase.

URATE IS FURTHER DEGRADED IN SOME ORGANISMS

The breakdown of purines proceeds further in some species (Figure 22-29). Mammals other than primates excrete *allantoin*, which is

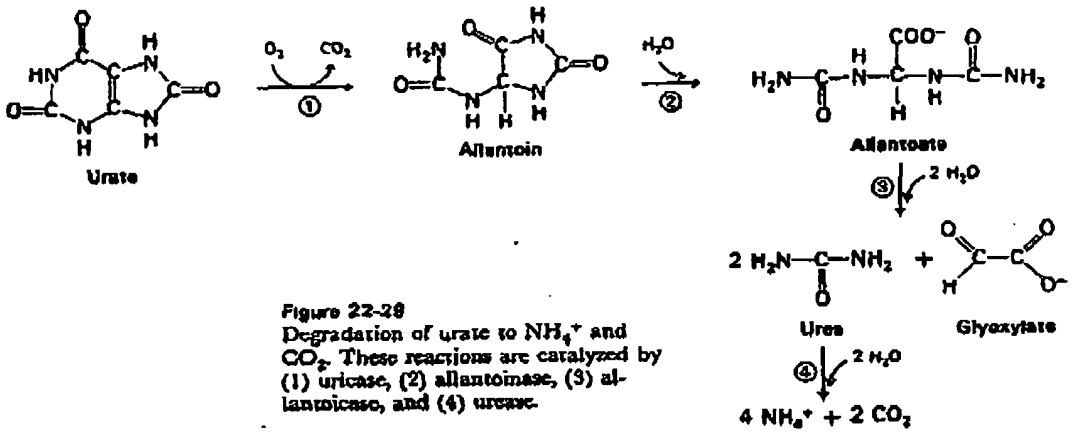
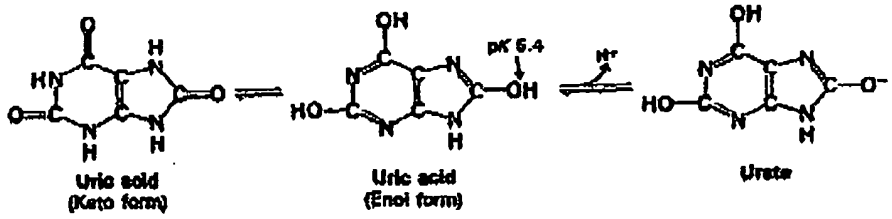


Figure 22-29
Degradation of urate to NH_4^+ and CO_2. These reactions are catalyzed by (1) uricase, (2) allantoinase, (3) allantoicase, and (4) urease.

formed by oxidation of urate. Teleost fish excrete *allantoate*, which is formed by hydration of allantoin. The degradation proceeds a step further in amphibians and most fish. Allantoate is hydrolyzed to two molecules of *urea* and one of *glyoxylate*. Finally, some marine invertebrates hydrolyze urea to NH_4^+ and CO_2. It seems likely that the enzymes catalyzing these reactions were progressively lost in the evolution of primates.

BIRDS AND TERRESTRIAL REPTILES EXCRETE URATE INSTEAD OF UREA TO CONSERVE WATER

In terrestrial reptiles and birds, urea is not the final product of amino nitrogen metabolism. These animals synthesize purines from their excess amino nitrogen and then degrade these purines to urate. *This circuitous path for the elimination of amino nitrogen serves a vital function: the conservation of water. Urate is the vehicle for the excretion of amino nitrogen because of its very low solubility at acid pH.* The pK_a of the most acidic group in uric acid is 5.4.



The acidic urine of birds and terrestrial reptiles consists of a paste of crystals of uric acid. Little water accompanies the excretion of these crystals. In contrast, the excretion of a comparable amount of highly soluble urea would be accompanied by a large efflux of water.

DEGRADATION OF PYRIMIDINES

The degradation of thymine (Figure 22-30) is illustrative of the breakdown of pyrimidines. Thymine is degraded to β-aminoisobutyrate, which is metabolized as though it were an α-amino acid. The amino group is removed by transamination to yield methylmalonate semialdehyde, which is converted into methylmalonyl CoA. The conversion of methylmalonyl CoA into succinyl CoA, the point of entry into the citric acid cycle, has already been discussed (p. 419).

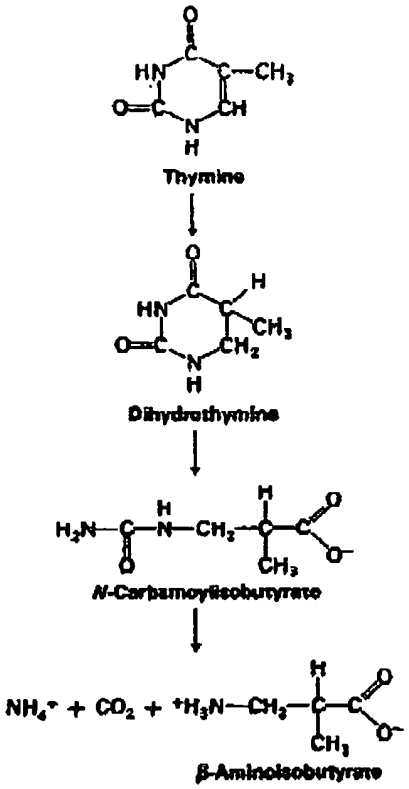


Figure 22-30
Degradation of thymine.

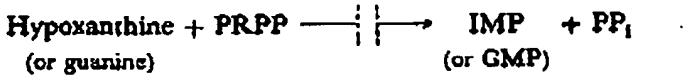
EXCESSIVE PRODUCTION OF URATE IS A CAUSE OF GOUT

Gout is a disease that affects the joints and leads to arthritis. The major biochemical feature of gout is an *elevated level of urate in the serum*. Inflammation of the joints is triggered by the *precipitation of sodium urate crystals*. Kidney disease may also occur because of the deposition of urate crystals in that organ. Gout primarily affects adult males. A vivid description of an acute attack of gout was given by Thomas Sydenham, an outstanding seventeenth-century English physician, who himself was afflicted with this disease:

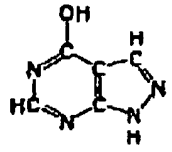
> The victim goes to bed and sleeps in good health. About two o'clock in the morning he is awakened by a severe pain in the great toe; more rarely in the heel, ankle or instep. This pain is like that of a dislocation, and yet the parts feel as if cold water were poured over them. Then follow chills and shivers, and a little fever. The pain, which was at first moderate, becomes more intense. With its intensity the chills and shivers increase. After a time this comes to its height, accommodating itself to the bones and ligaments of the tarsus and metatarsus. Now it is a violent stretching and tearing of the ligaments—now it is a gnawing pain and now a pressure and tightening. So exquisite and lively meanwhile is the feeling of the part affected, that it cannot bear the weight of the bedclothes nor the jar of a person walking in the room. The night is passed in torture, sleeplessness, turning of the part affected, and perpetual change of posture; the tossing about of the body being as incessant as the pain of the tortured joint, and being worse as the fit comes on.

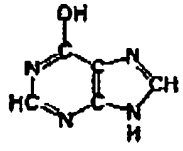
The biochemical lesion in most cases of gout has not been elucidated. It seems likely that gout is an expression of a variety of inborn errors of metabolism in which *excessive production of urate* is a common finding. Some patients with this abnormality have a par-

tial deficiency of *hypoxanthine-guanine phosphoribosyltransferase*, the enzyme that catalyzes the salvage synthesis of IMP and GMP.

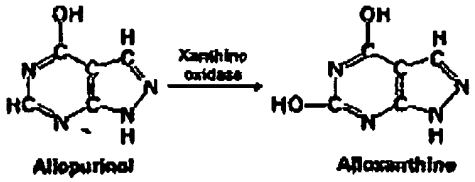


Deficiency of this enzyme leads to reduced synthesis of GMP and IMP by the salvage pathway and an increase in the level of PRPP. There is a *marked acceleration of purine biosynthesis by the de novo pathway*. A few patients with gout have an *abnormally high level of phosphoribosylpyrophosphate synthetase*. The allosteric control of this enzyme is impaired in these patients. This results in *excessive production of PRPP*, which in turn accelerates the rate of de novo synthesis of purines.

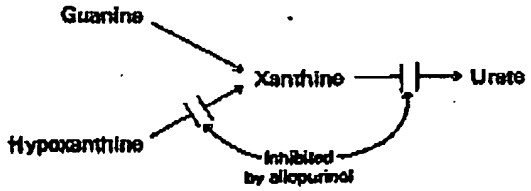

Allopurinol


Hypoxanthine

Allopurinol, an analog of hypoxanthine in which the positions of N7 and C8 are interchanged, is used to treat gout. The mechanism of action of allopurinol is very interesting: it acts *first as a substrate* and *then as an inhibitor* of xanthine oxidase. This enzyme hydroxylates allopurinol to *alloxanthine*, which then remains tightly bound to the active site. The molybdenum atom of xanthine oxidase is kept in the +4 oxidation state by the binding of alloxanthine instead of returning to the +6 oxidation state as it does in a normal catalytic cycle. This mode of action of allopurinol is an example of *suicide inhibition*, in which an enzyme converts a compound into a potent inhibitor that immediately inactivates the enzyme.



The synthesis of urate from hypoxanthine and xanthine decreases soon after the administration of allopurinol.



The serum concentrations of hypoxanthine and xanthine increase after administration of allopurinol, whereas that of urate drops. The formation of uric acid stones is virtually abolished by allopurinol, and there is some improvement in the arthritis. Also, *there is a decrease in the total rate of purine biosynthesis*. This inhibitory action of

allopurinol depends on its reaction with PRPP to form the ribonucleotide. Consequently, the level of PRPP, the limiting substrate in the de novo synthesis of purines, is lowered. Furthermore, allopurinol ribonucleotide inhibits the conversion of PRPP into phosphoribosylamine by amidophosphoribosyltransferase.

LESCH-NYHAN SYNDROME: SELF-MUTILATION, MENTAL RETARDATION, AND EXCESSIVE PRODUCTION OF URATE

A nearly total absence of hypoxanthine-guanine phosphoribosyltransferase has devastating consequences. The most striking expression of this inborn error of metabolism, called the *Lesch-Nyhan syndrome,* is *compulsive self-destructive behavior.* At age two or three, children with this disease begin to bite their fingers and lips. The tendency to self-mutilate is so extreme that it is necessary to protect these patients by such measures as wrapping their hands in gauze. Those afflicted also tend to be aggressive toward others. *Mental deficiency and spasticity* are other characteristics of the Lesch-Nyhan syndrome. Elevated levels of urate in the serum lead to the formation of stones early in life, followed by the symptoms of *gout* years later. The disease is inherited as a sex-linked recessive.

The biochemical consequences of the virtual absence of hypoxanthine-guanine phosphoribosyltransferase are an *overproduction of urate* and an *elevated concentration of PRPP.* Also, there is a marked increase in the rate of purine biosynthesis by the de novo pathway. The relationships between the absence of the transferase and the bizarre neurologic signs are an enigma. The brain may be very dependent on the salvage pathway for the synthesis of IMP and GMP. The normal level of hypoxanthine-guanine phosphoribosyltransferase is higher in the brain than in any other tissue. In contrast, the activity of the amidotransferase that catalyzes the committed step in the de novo pathway is rather low in the brain. Allopurinol is effective in diminishing urate synthesis in the Lesch-Nyhan syndrome. However, it has no effect on the rate of de novo synthesis of purines and it fails to alleviate the neurologic expressions of the disease. Patients with the Lesch-Nyhan syndrome do not convert allopurinol into the ribonucleotide because they lack hypoxanthine-guanine phosphoribosyltransferase. Hence, the administration of allopurinol does not lower the level of PRPP in these individuals and so de novo purine synthesis is not diminished.

The Lesch-Nyhan syndrome demonstrates that the salvage pathway for the synthesis of IMP and GMP is not gratuitous. The salvage pathway evidently serves a critical role that is not yet fully understood. Furthermore, the interplay between the de novo and salvage pathways of purine synthesis remains to be elucidated. Moreover, the Lesch-Nyhan syndrome reveals that abnormal behavior such as self-mutilation and extreme hostility can be caused by the absence of a single enzyme. This finding has important implications for the future development of psychiatry.

SUMMARY

The purine ring is assembled from a variety of precursors: glutamine, glycine, aspartate, methenyltetrahydrofolate, N^{10}-formyltetrahydrofolate, and CO_2. The committed step in the de novo synthesis of purine nucleotides is the formation of 5-phosphoribosylamine from PRPP and glutamine. The purine ring is attached to ribose phosphate during its assembly. The addition of glycine, followed by formylation, amination, and ring closure, yields 5-aminoimidazole ribonucleotide. This intermediate contains the completed five-membered ring of the purine skeleton. The addition of CO_2, the nitrogen atom of aspartate and a formyl group, followed by ring closure, yields inosinate (IMP), a purine ribonucleotide. AMP and GMP are formed from IMP. Purine ribonucleotides can also be synthesized by a salvage pathway in which a preformed base reacts directly with PRPP. Feedback inhibition of 5-phosphoribosyl-1-pyrophosphate synthetase and of glutamine-PRPP amidotransferase by purine nucleotides is important in regulating their biosynthesis.

The pyrimidine ring is assembled first and then linked to ribose phosphate to form a pyrimidine nucleotide, in contrast with the sequence in the de novo synthesis of purine nucleotides. PRPP is again the donor of the ribose phosphate moiety. The synthesis of the pyrimidine ring starts with the formation of carbamoylaspartate from carbamoyl phosphate and aspartate, a reaction catalyzed by aspartate transcarbamoylase. Dehydration, cyclization, and oxidation yield orotate, which reacts with PRPP to give orotidylate. Decarboxylation of this pyrimidine nucleotide yields UMP. CTP is then formed by amination of UTP. Pyrimidine biosynthesis in *E. coli* is regulated by feedback inhibition of aspartate transcarbamoylase, the enzyme that catalyzes the committed step. CTP inhibits and ATP stimulates this enzyme. Aspartate transcarbamoylase consists of separable regulatory and catalytic subunits. In mammalian cells, a single polypeptide chain contains the first three enzymes of pyrimidine biosynthesis.

Deoxyribonucleotides, the precursors of DNA, are formed by the reduction of ribonucleoside diphosphates. These conversions are catalyzed by ribonucleotide reductase. Electrons are transferred from NADPH to the sulfhydryl groups at the catalytic sites of this enzyme by thioredoxin or glutaredoxin. dTMP is formed by the methylation of dUMP. The one-carbon and electron donor in this reaction is N^5,N^{10}-methylenetetrahydrofolate, which is converted into dihydrofolate. In turn, tetrahydrofolate is regenerated by the reduction of dihydrofolate. Dihydrofolate reductase, which catalyzes this reaction, is inhibited by folate analogs such as aminopterin and amethopterin (methotrexate). These compounds are used as antitumor drugs.

APPENDIX B

C. glutamicum strain	Lysine Formation (g/l)
Control	12.50
Experimental (strong promoter introduced)	13.88

Appendix C

lalign output for USPN 6,797,509 SEQ ID NO:1 vs. USSN 09/602740 SEQ ID NO:1

[ISREC-Server] Date: Mon Mar 20 20:35:38 Europe/Zurich 2006

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resetting matrix to DNA ./wwwtmp/lalign/.4549.1.seq : 6995 nt

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ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
USPN 6,797,509 SEQ ID NO:1                          6995 nt vs.
USSN 09/602740 SEQ ID NO:1                           828 nt
scoring matrix: DNA, gap penalties: -14/-4
11.8% identity;          Global alignment score: -20548

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USSN   ------------------------------------------------------------


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USSN   ------------------------------------------------------------


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USSN   ------------------------------------------------------------


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USSN   ------------------------------------------------------------


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USSN   ------------------------------------------------------------


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USSN   ------------------------------------------------------------


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USSN   ------------------------------------------------------------


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USSN  ------------------------------------------------------------

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USSN  ------------------------------------------------------------

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USSN  ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN --

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USSN --

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USSN --

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USSN --

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USSN --

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USSN --

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

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USSN    ------------------------------------------------------------

              5770       5780       5790       5800       5810       5820
./wwwt CCTCCTCATTGGATCACCCACCACACAGCGAAATCACTTCCGTGAGGCTGACCGGTGCAA

USSN    ------------------------------------------------------------

              5830       5840       5850       5860       5870       5880
./wwwt GCGGCAGTACCTCGGTGGATTTGGCTGCAGGCTGGTTGGCGCGGAGGCTGAAAGTGCCTG
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USSN    ------------------------------------------------------------

              5890       5900       5910       5920       5930       5940
./wwwt TGATCCGCGAGGTGACAGATGCTCCCACCGTGCCAACCGATGAGTTTGGTACTCCACTGC

USSN    ------------------------------------------------------------

              5950       5960       5970       5980       5990       6000
./wwwt TGGCTATCCAGCGCCTGGAGATCGTTCGCACCACCGGCTCGATCATCATCACCATCTATG

USSN    ------------------------------------------------------------

              6010       6020       6030       6040       6050       6060
./wwwt ACGCTCATACCCTTCAGGTAGAGATGCCGGAATCCGGCAATGCCCCATCGCTGGTGGCTA

USSN    ------------------------------------------------------------

              6070       6080       6090       6100       6110       6120
./wwwt TTGGTCGTCGAAGTGAGTCCGACTGCTTGTCTGAGGAGCTTCGCCACATGGATCCAGATT
                                                :::::::::::::::::::::::::
USSN    ------------------------------------GAGGAGCTTCGCCACATGGATCCAGATT
                                                      10         20

              6130       6140       6150       6160       6170       6180
./wwwt TGGGCTACCAGCACGCACTATCCGGCTTGTCCAGCGTCAAGCTGGAAACCGTCTAAGGAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN    TGGGCTACCAGCACGCACTATCCGGCTTGTCCAGCGTCAAGCTGGAAACCGTCTAAGGAG
       30         40         50         60         70         80

              6190       6200       6210       6220       6230       6240
./wwwt AAATACAACACTATGGTTGATGTAGTACGCGCACGCGATACTGAAGATTTGGTTGCACAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN    AAATACAACACTATGGTTGATGTAGTACGCGCACGCGATACTGAAGATTTGGTTGCACAG
       90         100        110        120        130        140

              6250       6260       6270       6280       6290       6300
./wwwt GCTGCCTCCAAATTCATTGAGGTTGTTGAAGCAGCAACTGCCAATAATGGCACCGCACAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN    GCTGCCTCCAAATTCATTGAGGTTGTTGAAGCAGCAACTGCCAATAATGGCACCGCACAG
      150        160        170        180        190        200

              6310       6320       6330       6340       6350       6360
./wwwt GTAGTGCTCACCGGTGGTGGCGCCGGCATCAAGTTGCTGGAAAAGCTCAGCGTTGATGCG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN    GTAGTGCTCACCGGTGGTGGCGCCGGCATCAAGTTGCTGGAAAAGCTCAGCGTTGATGCG
      210        220        230        240        250        260

              6370       6380       6390       6400       6410       6420
./wwwt GCTGACCTTGCCTGGGATCGCATTCATGTGTTCTTCGGCGATGAGCGCAATGTCCCTGTC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN    GCTGACCTTGCCTGGGATCGCATTCATGTGTTCTTCGGCGATGAGCGCAATGTCCCTGTC
      270        280        290        300        310        320

              6430       6440       6450       6460       6470       6480
./wwwt AGTGATTCTGAGTCCAATGAGGGCCAGGCTCGTGAGGCACTGTTGTCCAAGGTTTCTATC
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         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN     AGTGATTCTGAGTCCAATGAGGGCCAGGCTCGTGAGGCACTGTTGTCCAAGGTTTCTATC
       330       340       350       360       370       380

              6490      6500      6510      6520      6530      6540
./wwwt   CCTGAAGCCAACATTCACGGATATGGTCTCGGCGACGTAGATCTTGCAGAGGCAGCCCGC
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN     CCTGAAGCCAACATTCACGGATATGGTCTCGGCGACGTAGATCTTGCAGAGGCAGCCCGC
       390       400       410       420       430       440

              6550      6560      6570      6580      6590      6600
./wwwt   GCTTACGAAGCTGTGTTGGATGAATTCGCACCAAACGGCTTTGATCTTCACCTGCTCGGC
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN     GCTTACGAAGCTGTGTTGGATGAATTCGCACCAAACGGCTTTGATCTTCACCTGCTCGGC
       450       460       470       480       490       500

              6610      6620      6630      6640      6650      6660
./wwwt   ATGGGTGGCGAAGGCCATATCAACTCCCTGTTCCCTCACACCGATGCAGTCAAGGAATCC
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN     ATGGGTGGCGAAGGCCATATCAACTCCCTGTTCCCTCACACCGATGCAGTCAAGGAATCC
       510       520       530       540       550       560

              6670      6680      6690      6700      6710      6720
./wwwt   TCCGCAAAGGTCATCGCGGTGTTTGATTCCCCTAAGCCTCCTTCAGAGCGTGCAACTCTA
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN     TCCGCAAAGGTCATCGCGGTGTTTGATTCCCCTAAGCCTCCTTCAGAGCGTGCAACTCTA
       570       580       590       600       610       620

              6730      6740      6750      6760      6770      6780
./wwwt   ACCCTTCCTGCGGTTCACTCCGCAAAGCGCGTGTGGTTGCTGGTTTCTGGTGCGGAGAAG
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN     ACCCTTCCTGCGGTTCACTCCGCAAAGCGCGTGTGGTTGCTGGTTTCTGGTGCGGAGAAG
       630       640       650       660       670       680

              6790      6800      6810      6820      6830      6840
./wwwt   GCTGAGGCAGCTGCGGCGATCGTCAACGGTGAGCCTGCTGTTGAGTGGCCTGCTGCTGGA
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN     GCTGAGGCAGCTGCGGCGATCGTCAACGGTGAGCCTGCTGTTGAGTGGCCTGCTGCTGGA
       690       700       710       720       730       740

              6850      6860      6870      6880      6890      6900
./wwwt   GCTACCGGATCTGAGGAAACCGTATTGTTCTTGGCTGATGATGCTGCAGGAAATCTCTAA
         ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
USSN     GCTACCGGATCTGAGGAAACCGTATTGTTCTTGGCTGATGATGCTGCAGGAAATCTCTAA
       750       760       770       780       790       800

              6910      6920      6930      6940      6950      6960
./wwwt   GCAGCGCCAGCTCTAACAAGAAGCTTTAACAAGAAGCTCTAACGAAAAGCACTAACAAAC
         ::::::::::::::::::::
USSN     GCAGCGCCAGCTCTAACAAG----------------------------------------
       810       820

              6970      6980      6990
./wwwt   TAATCCGGGTGCGAACCTTCATCTGAATCGATGGA

USSN     -----------------------------------
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